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**GROWTH-ASSOCIATED PROTEASE INHIBITOR HEAVY CHAIN PRECURSOR**

~~Serial~~ This application is a divisional application of U.S. application Serial No. 09/388,774 filed September 2, 1999, which is a divisional application of U.S. application Serial No. 09/074,579 filed May 7, 1998, issued December 14, 1999 as U.S. Patent No. 6,001,596, all of which applications and patents are hereby incorporated by reference herein.

**FIELD OF THE INVENTION**

This invention relates to nucleic acid and amino acid sequences of a growth-associated protease inhibitor heavy chain precursor and to the use of these sequences in the diagnosis, treatment, and prevention of reproductive, developmental, neoplastic, and immunological disorders.

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**BACKGROUND OF THE INVENTION**

Proteolytic processing is an essential component of normal cell growth, differentiation, remodeling, and homeostasis. The cleavage of peptide bonds within cells is necessary for the maturation of precursor proteins to their active form, the removal of signal sequences from targeted proteins, the degradation of incorrectly folded proteins, and the controlled turnover of peptides within the cell. Proteases participate in apoptosis, antigen presentation, inflammation, tissue remodeling during embryonic development, wound healing, and normal growth. They are necessary components of bacterial, parasitic, and viral invasion and replication within a host. Four principal categories of mammalian proteases have been identified based on active site structure, mechanism of action, and overall three-dimensional structure. (Beynon, R.J. and Bond, J.S. (1994) Proteolytic Enzymes: A Practical Approach, Oxford University Press, New York, NY, pp. 1-5.)

The serine proteases (SPs) are a large family of proteolytic enzymes that include the digestive enzymes, trypsin and chymotrypsin; components of the complement cascade and of the blood-clotting cascade; and enzymes that control the degradation and turnover of macromolecules of the extracellular matrix. SPs are so named because of the presence of a serine residue found in the active catalytic site for protein cleavage. The active site of all SP is composed of a triad of residues including the aforementioned serine, an aspartate, and a histidine residue. SPs have a wide range of substrate specificities and can be subdivided into subfamilies on the basis of these specificities. The main sub-families are trypases which cleave after arginine or lysine; aspases which cleave after aspartate; chymases which cleave after phenylalanine or leucine; metases which cleavage after methionine; and serases which cleave after serine.

The plasma inter- $\alpha$ -trypsin inhibitor family molecules are serine protease inhibitors (serpins) composed of a 240 kDa plasma protein complex of at least five different types of glycoproteins.

These glycoproteins consist of four heavy (H) chains and one 30 kDa light (L) chain named H1, H2, H3, H4, and L, and are independently synthesized and proteolytically processed from precursor proteins. (Daveau, M. et al. (1998) Arch. Biochem. Biophys. 350:315-323; and Salier, J.P. et al. (1992) Mamm. Genome 2:233-239.) The plasma inter- $\alpha$ -trypsin inhibitor light chains have sequence similarity to the Kunitz trypsin inhibitors which appear to be present in all vertebrates. (Salier, J.P. (1990) Trends Biochem. Sci. 15:435-439.) Some examples of the Kunitz trypsin inhibitors are tissue factor pathway inhibitor, which regulates tissue factor-induced coagulation, and protease nexin-2, which regulates serum coagulation factor XIa. (Broze, G.J. (1995) Annu. Rev. Med. 46:103-112; and Wagner, S.L. et al. (1993) Brain Res. 626:90-98.) The heavy chain precursors encode a signal peptide sequence and the mature chain. Other plasma inter- $\alpha$ -trypsin inhibitor heavy chains have been described in human and rodents. (Bourguignon, J. et al. (1993) Eur. J. Biochem. 212:771-776; Salier, 1992, *supra*; and Salier, J.P. (1996) Biochem. J. 315:1-9.) Proteases and protease inhibitory molecules may contain amino acid sequence motifs which determine protein-protein interactions, such as the potential metal-binding site of von Willebrand factor type A3 (vWFA3) motif, glycine-amino acid-serine-amino acid-serine. This motif is also required for ligand interaction in the homologous I-type domains of integrins CR3 and LFA-1. (Huizinga, E.G. (1997) Structure 5:1147-1156.)

The expression of the rat plasma inter- $\alpha$ -trypsin inhibitor genes is regulated by inflammation in vivo. The genes are predominantly expressed in the rat liver, but H2 and H3 mRNA is also present in brain, intestine, and stomach. (Daveau, *supra*.)

Protease inhibitors play a major role in the regulation of the activity and effect of proteases. They have been shown to control pathogenesis in animal models of proteolytic disorders and in the treatment of HIV. (Murphy, G. (1991) Agents Actions Suppl. 35:69-76; and Pakyz, A. and Isreal, D. (1997) J. Am. Pharm. Assoc. (Wash.) NS37:543-551.)

The discovery of a new growth-associated protease inhibitor heavy chain precursor and the polynucleotides encoding it satisfies a need in the art by providing new compositions which are useful in the diagnosis, treatment, and prevention of reproductive, developmental, neoplastic, and immunological disorders.

## SUMMARY OF THE INVENTION

The invention is based on the discovery of a new human growth-associated protease inhibitor heavy chain precursor (GAPIP), the polynucleotides encoding GAPIP, and the use of these compositions for the diagnosis, treatment, or prevention of reproductive, developmental, neoplastic,

and immunological disorders.

The invention features a substantially purified polypeptide comprising the amino acid sequence of SEQ ID NO:1 or a fragment of SEQ ID NO:1.

The invention further provides a substantially purified variant having at least 90% amino acid sequence identity to the amino acid sequence of SEQ ID NO:1 or a fragment of SEQ ID NO:1. The invention also provides an isolated and purified polynucleotide encoding the polypeptide comprising the sequence of SEQ ID NO:1 or a fragment of SEQ ID NO:1. The invention also includes an isolated and purified polynucleotide variant having at least 90% polynucleotide sequence identity to the polynucleotide encoding the polypeptide comprising the amino acid sequence of SEQ ID NO:1 or a fragment of SEQ ID NO:1.

The invention further provides an isolated and purified polynucleotide which hybridizes under stringent conditions to the polynucleotide encoding the polypeptide comprising the amino acid sequence of SEQ ID NO:1 or a fragment of SEQ ID NO:1, as well as an isolated and purified polynucleotide which is complementary to the polynucleotide encoding the polypeptide comprising the amino acid sequence of SEQ ID NO:1 or a fragment of SEQ ID NO:1.

The invention also provides an isolated and purified polynucleotide comprising the polynucleotide sequence of SEQ ID NO:2 or a fragment of SEQ ID NO:2, and an isolated and purified polynucleotide variant having at least 90% polynucleotide sequence identity to the polynucleotide comprising the polynucleotide sequence of SEQ ID NO:2 or a fragment of SEQ ID NO:2. The invention also provides an isolated and purified polynucleotide having a sequence complementary to the polynucleotide comprising the polynucleotide sequence of SEQ ID NO:2 or a fragment of SEQ ID NO:2.

The invention further provides an expression vector comprising at least a fragment of the polynucleotide encoding the polypeptide comprising the sequence of SEQ ID NO:1 or a fragment of SEQ ID NO:1. In another aspect, the expression vector is contained within a host cell.

The invention also provides a method for producing a polypeptide comprising the amino acid sequence of SEQ ID NO:1 or a fragment of SEQ ID NO:1, the method comprising the steps of: (a) culturing the host cell comprising an expression vector containing at least a fragment of a polynucleotide encoding the polypeptide comprising the amino acid sequence of SEQ ID NO:1 or a fragment of SEQ ID NO:1 under conditions suitable for the expression of the polypeptide; and (b) recovering the polypeptide from the host cell culture.

The invention also provides a pharmaceutical composition comprising a substantially purified polypeptide having the sequence of SEQ ID NO:1 or a fragment of SEQ ID NO:1 in

conjunction with a suitable pharmaceutical carrier.

The invention further includes a purified antibody which binds to a polypeptide comprising the sequence of SEQ ID NO:1 or a fragment of SEQ ID NO:1, as well as a purified agonist and a purified antagonist of the polypeptide.

5 The invention also provides a method for treating or preventing a reproductive disorder, the method comprising administering to a subject in need of such treatment an effective amount of a pharmaceutical composition comprising substantially purified polypeptide having the amino acid sequence of SEQ ID NO:1 or a fragment of SEQ ID NO:1.

10 The invention also provides a method for treating or preventing a developmental disorder, the method comprising administering to a subject in need of such treatment an effective amount of a pharmaceutical composition comprising substantially purified polypeptide having the amino acid sequence of SEQ ID NO:1 or a fragment of SEQ ID NO:1.

15 The invention also provides a method for treating or preventing a neoplastic disorder, the method comprising administering to a subject in need of such treatment an effective amount of an antagonist of the polypeptide having the amino acid sequence of SEQ ID NO:1 or a fragment of SEQ ID NO:1.

20 The invention also provides a method for treating or preventing an immunological disorder, the method comprising administering to a subject in need of such treatment an effective amount of an antagonist of the polypeptide having the amino acid sequence of SEQ ID NO:1 or a fragment of SEQ ID NO:1.

25 The invention also provides a method for detecting a polynucleotide encoding a polypeptide comprising the amino acid sequence of SEQ ID NO: 1 or a fragment of SEQ ID NO: 1 in a biological sample containing nucleic acids, the method comprising the steps of: (a) hybridizing the complement of the polynucleotide encoding the polypeptide comprising the amino acid sequence of SEQ ID NO:1 or a fragment of SEQ ID NO:1 to at least one of the nucleic acids of the biological sample, thereby forming a hybridization complex; and (b) detecting the hybridization complex, wherein the presence of the hybridization complex correlates with the presence of a polynucleotide encoding the polypeptide comprising the amino acid sequence of SEQ ID NO:1 or a fragment of SEQ ID NO:1 in the biological sample. In one aspect, the nucleic acids of the biological sample are amplified by the 30 polymerase chain reaction prior to the hybridizing step.

**BRIEF DESCRIPTION OF THE FIGURES**

Figures 1A, 1B, 1C, 1D, 1E, 1F, 1G, 1H, 1I, and 1J show the amino acid sequence (SEQ ID NO:1) and nucleic acid sequence (SEQ ID NO:2) of GAPIP. The alignment was produced using MacDNASIS PRO software (Hitachi Software Engineering Co. Ltd., San Bruno, CA).

5 Figures 2A, 2B, 2C, 2D, 2E, 2F, and 2G show the amino acid sequence alignments among GAPIP (688183; SEQ ID NO:1), human pre-inter- $\alpha$ -trypsin inhibitor (GI 33985; SEQ ID NO:3), human pre-inter- $\alpha$ -trypsin inhibitor heavy chain H1 (GI 33989; SEQ ID NO:4), and human pre-inter- $\alpha$ -trypsin inhibitor heavy chain H3 (GI 288563; SEQ ID NO:5), produced using the multisequence alignment program of LASERGENE software (DNASTAR Inc, Madison WI).

10 Figure 3 shows the amino acid sequence phylogenetic tree among GAPIP (688183; SEQ ID NO:1), human pre-inter- $\alpha$ -trypsin inhibitor (GI 33985; SEQ ID NO:3), human pre-inter- $\alpha$ -trypsin inhibitor heavy chain H1 (GI 33989; SEQ ID NO:4), and human pre-inter- $\alpha$ -trypsin inhibitor heavy chain H3 (GI 288563; SEQ ID NO:5), produced using the multisequence alignment program of LASERGENE software (DNASTAR Inc).

**DESCRIPTION OF THE INVENTION**

Before the present proteins, nucleotide sequences, and methods are described, it is understood that this invention is not limited to the particular methodology, protocols, cell lines, vectors, and reagents described, as these may vary. It is also to be understood that the terminology used herein is for the purpose of describing particular embodiments only, and is not intended to limit the scope of the present invention which will be limited only by the appended claims.

It must be noted that as used herein and in the appended claims, the singular forms "a," "an," and "the" include plural reference unless the context clearly dictates otherwise. Thus, for example, a reference to "a host cell" includes a plurality of such host cells, and a reference to "an antibody" is a reference to one or more antibodies and equivalents thereof known to those skilled in the art, and so forth.

Unless defined otherwise, all technical and scientific terms used herein have the same meanings as commonly understood by one of ordinary skill in the art to which this invention belongs. Although any methods and materials similar or equivalent to those described herein can be used in the practice or testing of the present invention, the preferred methods, devices, and materials are now described. All publications mentioned herein are cited for the purpose of describing and disclosing the cell lines, vectors, and methodologies which are reported in the publications and which might be used in connection with the invention. Nothing herein is to be construed as an admission that the invention is not entitled to antedate such disclosure by virtue of prior invention.

**DEFINITIONS**

"GAPIP," as used herein, refers to the amino acid sequences of substantially purified GAPIP obtained from any species, particularly a mammalian species, including bovine, ovine, porcine, murine, equine, and preferably the human species, from any source, whether natural, synthetic, semi-synthetic, or recombinant.

The term "agonist," as used herein, refers to a molecule which, when bound to GAPIP, increases or prolongs the duration of the effect of GAPIP. Agonists may include proteins, nucleic acids, carbohydrates, or any other molecules which bind to and modulate the effect of GAPIP.

An "allelic variant," as this term is used herein, is an alternative form of the gene encoding GAPIP. Allelic variants may result from at least one mutation in the nucleic acid sequence and may result in altered mRNAs or in polypeptides whose structure or function may or may not be altered. Any given natural or recombinant gene may have none, one, or many allelic forms. Common mutational changes which give rise to allelic variants are generally ascribed to natural deletions, additions, or substitutions of nucleotides. Each of these types of changes may occur alone, or in combination with the others, one or more times in a given sequence.

"Altered" nucleic acid sequences encoding GAPIP, as described herein, include those sequences with deletions, insertions, or substitutions of different nucleotides, resulting in a polynucleotide the same as GAPIP or a polypeptide with at least one functional characteristic of GAPIP. Included within this definition are polymorphisms which may or may not be readily detectable using a particular oligonucleotide probe of the polynucleotide encoding GAPIP, and improper or unexpected hybridization to allelic variants, with a locus other than the normal chromosomal locus for the polynucleotide sequence encoding GAPIP. The encoded protein may also be "altered," and may contain deletions, insertions, or substitutions of amino acid residues which produce a silent change and result in a functionally equivalent GAPIP. Deliberate amino acid substitutions may be made on the basis of similarity in polarity, charge, solubility, hydrophobicity, hydrophilicity, and/or the amphipathic nature of the residues, as long as the biological or immunological activity of GAPIP is retained. For example, negatively charged amino acids may include aspartic acid and glutamic acid, positively charged amino acids may include lysine and arginine, and amino acids with uncharged polar head groups having similar hydrophilicity values may include leucine, isoleucine, and valine; glycine and alanine; asparagine and glutamine; serine and threonine; and phenylalanine and tyrosine.

The terms "amino acid" or "amino acid sequence," as used herein, refer to an oligopeptide, peptide, polypeptide, or protein sequence, or a fragment of any of these, and to naturally occurring or

synthetic molecules. In this context, "fragments," "immunogenic fragments," or "antigenic fragments" refer to fragments of GAPIP which are preferably about 5 to about 15 amino acids in length, most preferably 14 amino acids, and which retain some biological activity or immunological activity of GAPIP. Where "amino acid sequence" is recited herein to refer to an amino acid sequence of a naturally occurring protein molecule, "amino acid sequence" and like terms are not meant to limit the amino acid sequence to the complete native amino acid sequence associated with the recited protein molecule.

"Amplification," as used herein, relates to the production of additional copies of a nucleic acid sequence. Amplification is generally carried out using polymerase chain reaction (PCR) technologies well known in the art. (See, e.g., Dieffenbach, C.W. and G.S. Dveksler (1995) PCR Primer, a Laboratory Manual, Cold Spring Harbor Press, Plainview, NY, pp.1-5.)

The term "antagonist," as it is used herein, refers to a molecule which, when bound to GAPIP, decreases the amount or the duration of the effect of the biological or immunological activity of GAPIP. Antagonists may include proteins, nucleic acids, carbohydrates, antibodies, or any other molecules which decrease the effect of GAPIP.

As used herein, the term "antibody" refers to intact molecules as well as to fragments thereof, such as Fab, F(ab')<sub>2</sub>, and Fv fragments, which are capable of binding the epitopic determinant. Antibodies that bind GAPIP polypeptides can be prepared using intact polypeptides or using fragments containing small peptides of interest as the immunizing antigen. The polypeptide or oligopeptide used to immunize an animal (e.g., a mouse, a rat, or a rabbit) can be derived from the translation of RNA, or synthesized chemically, and can be conjugated to a carrier protein if desired. Commonly used carriers that are chemically coupled to peptides include bovine serum albumin, thyroglobulin, and keyhole limpet hemocyanin (KLH). The coupled peptide is then used to immunize the animal.

The term "antigenic determinant," as used herein, refers to that fragment of a molecule (i.e., an epitope) that makes contact with a particular antibody. When a protein or a fragment of a protein is used to immunize a host animal, numerous regions of the protein may induce the production of antibodies which bind specifically to antigenic determinants (given regions or three-dimensional structures on the protein). An antigenic determinant may compete with the intact antigen (i.e., the immunogen used to elicit the immune response) for binding to an antibody.

The term "antisense," as used herein, refers to any composition containing a nucleic acid sequence which is complementary to the "sense" strand of a specific nucleic acid sequence. Antisense molecules may be produced by any method including synthesis or transcription. Once

introduced into a cell, the complementary nucleotides combine with natural sequences produced by the cell to form duplexes and to block either transcription or translation. The designation "negative" can refer to the antisense strand, and the designation "positive" can refer to the sense strand.

As used herein, the term "biologically active," refers to a protein having structural, regulatory, or biochemical functions of a naturally occurring molecule. Likewise, "immunologically active" refers to the capability of the natural, recombinant, or synthetic GAPIP, or of any oligopeptide thereof, to induce a specific immune response in appropriate animals or cells and to bind with specific antibodies.

The terms "complementary" or "complementarity," as used herein, refer to the natural binding of polynucleotides under permissive salt and temperature conditions by base pairing. For example, the sequence "A-G-T" binds to the complementary sequence "T-C-A." Complementarity between two single-stranded molecules may be "partial," such that only some of the nucleic acids bind, or it may be "complete," such that total complementarity exists between the single stranded molecules. The degree of complementarity between nucleic acid strands has significant effects on the efficiency and strength of the hybridization between the nucleic acid strands. This is of particular importance in amplification reactions, which depend upon binding between nucleic acids strands, and in the design and use of peptide nucleic acid (PNA) molecules.

A "composition comprising a given polynucleotide sequence" or a "composition comprising a given amino acid sequence," as these terms are used herein, refer broadly to any composition containing the given polynucleotide or amino acid sequence. The composition may comprise a dry formulation, an aqueous solution, or a sterile composition. Compositions comprising polynucleotide sequences encoding GAPIP or fragments of GAPIP may be employed as hybridization probes. The probes may be stored in freeze-dried form and may be associated with a stabilizing agent such as a carbohydrate. In hybridizations, the probe may be deployed in an aqueous solution containing salts, e.g., NaCl, detergents, e.g., sodium dodecyl sulfate (SDS), and other components, e.g., Denhardt's solution, dry milk, salmon sperm DNA, etc.

"Consensus sequence," as used herein, refers to a nucleic acid sequence which has been resequenced to resolve uncalled bases, extended using XL-PCR (Applied Biosystems, Foster City CA) in the 5' and/or the 3' direction, and resequenced, or which has been assembled from the overlapping sequences of more than one Incyte Clone using a computer program for fragment assembly, such as the GELVIEW Fragment Assembly system (GCG, Madison, WI). Some sequences have been both extended and assembled to produce the consensus sequence.

As used herein, the term "correlates with expression of a polynucleotide" indicates that the

detection of the presence of nucleic acids, the same or related to a nucleic acid sequence encoding GAPIP, by Northern analysis is indicative of the presence of nucleic acids encoding GAPIP in a sample, and thereby correlates with expression of the transcript from the polynucleotide encoding GAPIP.

5 A “deletion,” as the term is used herein, refers to a change in the amino acid or nucleotide sequence that results in the absence of one or more amino acid residues or nucleotides.

The term “derivative,” as used herein, refers to the chemical modification of a polypeptide sequence, or a polynucleotide sequence. Chemical modifications of a polynucleotide sequence can include, for example, replacement of hydrogen by an alkyl, acyl, or amino group. A derivative 10 polynucleotide encodes a polypeptide which retains at least one biological or immunological function of the natural molecule. A derivative polypeptide is one modified by glycosylation, pegylation, or any similar process that retains at least one biological or immunological function of the polypeptide from which it was derived.

The term “similarity,” as used herein, refers to a degree of complementarity. There may be partial similarity or complete similarity. The word “identity” may substitute for the word “similarity.” A partially complementary sequence that at least partially inhibits an identical sequence from hybridizing to a target nucleic acid is referred to as “substantially similar.” The inhibition of hybridization of the completely complementary sequence to the target sequence may be examined using a hybridization assay (Southern or Northern blot, solution hybridization, and the like) under conditions of reduced stringency. A substantially similar sequence or hybridization probe will compete for and inhibit the binding of a completely similar (identical) sequence to the target sequence under conditions of reduced stringency. This is not to say that conditions of reduced stringency are such that non-specific binding is permitted, as reduced stringency conditions require that the binding of two sequences to one another be a specific (i.e., a selective) interaction. The 25 absence of non-specific binding may be tested by the use of a second target sequence which lacks even a partial degree of complementarity (e.g., less than about 30% similarity or identity). In the absence of non-specific binding, the substantially similar sequence or probe will not hybridize to the second non-complementary target sequence.

The phrases “percent identity” or “% identity” refer to the percentage of sequence similarity 30 found in a comparison of two or more amino acid or nucleic acid sequences. Percent identity can be determined electronically, e.g., by using the MEGALIGN program (DNASTAR, Inc.). The MEGALIGN program can create alignments between two or more sequences according to different methods, e.g., the clustal method. (See, e.g., Higgins, D.G. and P.M. Sharp (1988) Gene 73:237-

244.) The clustal algorithm groups sequences into clusters by examining the distances between all pairs. The clusters are aligned pairwise and then in groups. The percentage similarity between two amino acid sequences, e.g., sequence A and sequence B, is calculated by dividing the length of sequence A, minus the number of gap residues in sequence A, minus the number of gap residues in sequence B, into the sum of the residue matches between sequence A and sequence B, times one hundred. Gaps of low or of no similarity between the two amino acid sequences are not included in determining percentage similarity. Percent identity between nucleic acid sequences can also be counted or calculated by other methods known in the art, e.g., the Jotun Hein method. (See, e.g., Hein, J. (1990) Methods Enzymol. 183:626-645.) Identity between sequences can also be determined by other methods known in the art, e.g., by varying hybridization conditions.

"Human artificial chromosomes" (HACs), as described herein, are linear microchromosomes which may contain DNA sequences of about 6 kb to 10 Mb in size, and which contain all of the elements required for stable mitotic chromosome segregation and maintenance. (See, e.g., Harrington, J.J. et al. (1997) Nat Genet. 15:345-355.)

The term "humanized antibody," as used herein, refers to antibody molecules in which the amino acid sequence in the non-antigen binding regions has been altered so that the antibody more closely resembles a human antibody, and still retains its original binding ability.

"Hybridization," as the term is used herein, refers to any process by which a strand of nucleic acid binds with a complementary strand through base pairing.

As used herein, the term "hybridization complex" refers to a complex formed between two nucleic acid sequences by virtue of the formation of hydrogen bonds between complementary bases. A hybridization complex may be formed in solution (e.g., C<sub>0</sub>t or R<sub>0</sub>t analysis) or formed between one nucleic acid sequence present in solution and another nucleic acid sequence immobilized on a solid support (e.g., paper, membranes, filters, chips, pins or glass slides, or any other appropriate substrate to which cells or their nucleic acids have been fixed).

The words "insertion" or "addition," as used herein, refer to changes in an amino acid or nucleotide sequence resulting in the addition of one or more amino acid residues or nucleotides, respectively, to the sequence found in the naturally occurring molecule.

"Immune response" can refer to conditions associated with inflammation, trauma, immune disorders, or infectious or genetic disease, etc. These conditions can be characterized by expression of various factors, e.g., cytokines, chemokines, and other signaling molecules, which may affect cellular and systemic defense systems.

The term "microarray," as used herein, refers to an arrangement of distinct polynucleotides

arrayed on a substrate, e.g., paper, nylon or any other type of membrane, filter, chip, glass slide, or any other suitable solid support.

The terms "element" or "array element" as used herein in a microarray context, refer to hybridizable polynucleotides arranged on the surface of a substrate.

5 The term "modulate," as it appears herein, refers to a change in the activity of GAPIP. For example, modulation may cause an increase or a decrease in protein activity, binding characteristics, or any other biological, functional, or immunological properties of GAPIP.

10 The phrases "nucleic acid" or "nucleic acid sequence," as used herein, refer to a nucleotide, oligonucleotide, polynucleotide, or any fragment thereof. These phrases also refer to DNA or RNA of genomic or synthetic origin which may be single-stranded or double-stranded and may represent the sense or the antisense strand, to peptide nucleic acid (PNA), or to any DNA-like or RNA-like material. In this context, "fragments" refers to those nucleic acid sequences which, when translated, would produce polypeptides retaining some functional characteristic, e.g., antigenicity, or structural domain characteristic, e.g., ATP-binding site, of the full-length polypeptide.

15 The terms "operably associated" or "operably linked," as used herein, refer to functionally related nucleic acid sequences. A promoter is operably associated or operably linked with a coding sequence if the promoter controls the translation of the encoded polypeptide. While operably associated or operably linked nucleic acid sequences can be contiguous and in the same reading frame, certain genetic elements, e.g., repressor genes, are not contiguously linked to the sequence encoding the polypeptide but still bind to operator sequences that control expression of the polypeptide.

20 The term "oligonucleotide," as used herein, refers to a nucleic acid sequence of at least about 6 nucleotides to 60 nucleotides, preferably about 15 to 30 nucleotides, and most preferably about 20 to 25 nucleotides, which can be used in PCR amplification or in a hybridization assay or microarray. As used herein, the term "oligonucleotide" is substantially equivalent to the terms "amplimer,"

25 "primer," "oligomer," and "probe," as these terms are commonly defined in the art.

30 "Peptide nucleic acid" (PNA), as used herein, refers to an antisense molecule or anti-gene agent which comprises an oligonucleotide of at least about 5 nucleotides in length linked to a peptide backbone of amino acid residues ending in lysine. The terminal lysine confers solubility to the composition. PNAs preferentially bind complementary single stranded DNA or RNA and stop transcript elongation, and may be pegylated to extend their lifespan in the cell. (See, e.g., Nielsen, P.E. et al. (1993) Anticancer Drug Des. 8:53-63.)

The term "sample," as used herein, is used in its broadest sense. A biological sample

suspected of containing nucleic acids encoding GAPIP, or fragments thereof, or GAPIP itself, may comprise a bodily fluid; an extract from a cell, chromosome, organelle, or membrane isolated from a cell; a cell; genomic DNA, RNA, or cDNA, in solution or bound to a solid support; a tissue; a tissue print; etc.

5 As used herein, the terms “specific binding” or “specifically binding” refer to that interaction between a protein or peptide and an agonist, an antibody, or an antagonist. The interaction is dependent upon the presence of a particular structure of the protein, e.g., the antigenic determinant or epitope, recognized by the binding molecule. For example, if an antibody is specific for epitope “A,” the presence of a polypeptide containing the epitope A, or the presence of free unlabeled A, in a  
10 reaction containing free labeled A and the antibody will reduce the amount of labeled A that binds to the antibody.

As used herein, the term “stringent conditions” refers to conditions which permit hybridization between polynucleotides and the claimed polynucleotides. Stringent conditions can be defined by salt concentration, the concentration of organic solvent (e.g., formamide), temperature, and other conditions well known in the art. In particular, stringency can be increased by reducing the concentration of salt, increasing the concentration of formamide, or raising the hybridization temperature.

For example, stringent salt concentration will ordinarily be less than about 750 mM NaCl and 75 mM trisodium citrate, preferably less than about 500 mM NaCl and 50 mM trisodium citrate, and most preferably less than about 250 mM NaCl and 25 mM trisodium citrate. Low stringency hybridization can be obtained in the absence of organic solvent, e.g., formamide, while high stringency hybridization can be obtained in the presence of at least about 35% formamide, and most preferably at least about 50% formamide. Stringent temperature conditions will ordinarily include temperatures of at least about 30°C, more preferably of at least about 37°C, and most preferably of at  
25 least about 42°C. Varying additional parameters, such as hybridization time, the concentration of detergent, e.g., sodium dodecyl sulfate (SDS), and the inclusion or exclusion of carrier DNA, are well known to those skilled in the art. Various levels of stringency are accomplished by combining these various conditions as needed. In a preferred embodiment, hybridization will occur at 30°C in 750 mM NaCl, 75 mM trisodium citrate, and 1% SDS. In a more preferred embodiment, hybridization will  
30 occur at 37°C in 500 mM NaCl, 50 mM trisodium citrate, 1% SDS, 35% formamide, and 100 µg/ml denatured salmon sperm DNA (ssDNA). In a most preferred embodiment, hybridization will occur at 42°C in 250 mM NaCl, 25 mM trisodium citrate, 1% SDS, 50 % formamide, and 200 µg/ml ssDNA. Useful variations on these conditions will be readily apparent to those skilled in the art.

The washing steps which follow hybridization can also vary in stringency. Wash stringency conditions can be defined by salt concentration and by temperature. As above, wash stringency can be increased by decreasing salt concentration or by increasing temperature. For example, stringent salt concentration for the wash steps will preferably be less than about 30 mM NaCl and 3 mM trisodium citrate, and most preferably less than about 15 mM NaCl and 1.5 mM trisodium citrate.

5 Stringent temperature conditions for the wash steps will ordinarily include temperature of at least about 25°C, more preferably of at least about 42°C, and most preferably of at least about 68°C. In a preferred embodiment, wash steps will occur at 25°C in 30 mM NaCl, 3 mM trisodium citrate, and 0.1% SDS. In a more preferred embodiment, wash steps will occur at 42°C in 15 mM NaCl, 1.5 mM trisodium citrate, and 0.1% SDS. In a most preferred embodiment, wash steps will occur at 68°C in 10 15 mM NaCl, 1.5 mM trisodium citrate, and 0.1% SDS. Additional variations on these conditions will be readily apparent to those skilled in the art.

The term "substantially purified," as used herein, refers to nucleic acid or amino acid sequences that are removed from their natural environment and are isolated or separated, and are at least about 60% free, preferably about 75% free, and most preferably about 90% free from other components with which they are naturally associated.

A "substitution," as used herein, refers to the replacement of one or more amino acids or nucleotides by different amino acids or nucleotides, respectively.

"Transformation," as defined herein, describes a process by which exogenous DNA enters and changes a recipient cell. Transformation may occur under natural or artificial conditions according to various methods well known in the art, and may rely on any known method for the insertion of foreign nucleic acid sequences into a prokaryotic or eukaryotic host cell. The method for transformation is selected based on the type of host cell being transformed and may include, but is not limited to, viral infection, electroporation, heat shock, lipofection, and particle bombardment.

25 The term "transformed" cells includes stably transformed cells in which the inserted DNA is capable of replication either as an autonomously replicating plasmid or as part of the host chromosome, as well as transiently transformed cells which express the inserted DNA or RNA for limited periods of time.

A "variant" of GAPIP, as used herein, refers to an amino acid sequence that is altered by one or more amino acids. The variant may have "conservative" changes, wherein a substituted amino acid has similar structural or chemical properties (e.g., replacement of leucine with isoleucine). More rarely, a variant may have "nonconservative" changes (e.g., replacement of glycine with tryptophan). Analogous minor variations may also include amino acid deletions or insertions, or both. Guidance

in determining which amino acid residues may be substituted, inserted, or deleted without abolishing biological or immunological activity may be found using computer programs well known in the art, for example, LASERGENE software.

5    **THE INVENTION**

The invention is based on the discovery of a new human growth-associated protease inhibitor heavy chain precursor (GAPIP), the polynucleotides encoding GAPIP, and the use of these compositions for the diagnosis, treatment, or prevention of reproductive, developmental, neoplastic, and immunological disorders.

10      Nucleic acids encoding the GAPIP of the present invention were first identified in Incyte Clone 688183 from the uterus cDNA library (UTRSNOT02) using a computer search, e.g., BLAST, for amino acid sequence alignments. A consensus sequence, SEQ ID NO:2, was derived from the following overlapping and/or extended nucleic acid sequences: Incyte Clones 688183 (UTRSNOT02), 3043969 (HEAANOT01), 3112673 (BRSTNOT17), 3052595 (LNODNOT08), 789100 (PROSTUT03), 785182 (PROSNOT05), 1505061 and 1505717 (BRAITUT07), 1794195 and 1795083 (PROSTUT05), 2125590 (BRSTNOT07), 1558218 (SPLNNOT04), 1361072 (LUNGNOT12), and 1964439 (BRSTNOT04).

In one embodiment, the invention encompasses a polypeptide comprising the amino acid sequence of SEQ ID NO:1, as shown in Figures 1A, 1B, 1C, 1D, 1E, 1F, 1G, 1H, 1I, and 1J. GAPIP is 942 amino acids in length and has eight potential N-glycosylation sites at residues N97, N127, N231, N421, N508, N776, N795, and N862; twelve potential casein kinase II phosphorylation sites at residues S17, S28, T112, T129, S158, S269, S354, T410, T581, S592, T676, and S754; two potential glycosaminoglycan attachment sites at residues S213 and S391; seventeen potential protein kinase C phosphorylation sites at residues S55, S70, T112, S175, S182, S213, S337, S354, T416, T458, S535, S559, T581, S611, S620, S651, and T880; one potential tyrosine kinase phosphorylation site at residue Y919; a potential signal peptide sequence from M1 to C14; and a vWFA3 domain, which contains the potential metal-binding site glycine-amino acid-serine-amino acid-serine, from N295 to N440. As shown in Figures 2A, 2B, 2C, 2D, 2E, 2F, and 2G, GAPIP has chemical and structural similarity with human pre-inter- $\alpha$ -trypsin inhibitor (GI 33985; SEQ ID NO:3), human pre-inter- $\alpha$ -trypsin inhibitor heavy chain H1 (GI 33989; SEQ ID NO:4), and human pre-inter- $\alpha$ -trypsin inhibitor heavy chain H3 (GI 288563; SEQ ID NO:5). In particular, GAPIP and human pre-inter- $\alpha$ -trypsin inhibitor share 28% identity, one potential N-glycosylation site, four potential casein kinase II phosphorylation sites, four potential protein kinase C phosphorylation sites, the potential signal

peptide sequence, and the vWFA3 potential metal-binding site glycine-amino acid-serine-amino acid-serine. In addition, GAPIP and human pre-inter- $\alpha$ -trypsin inhibitor heavy chains H1 and H3 share 27% and 23 % identity, respectively, one potential N-glycosylation site, four potential casein kinase II phosphorylation sites, five potential protein kinase C phosphorylation sites, the potential signal peptide sequence, and the vWFA3 potential metal-binding site glycine-amino acid-serine-amino acid-serine. As illustrated by Figure 3, GAPIP and human pre-inter- $\alpha$ -trypsin inhibitor heavy chains share a common phylogenetic heritage. A fragment of SEQ ID NO:2 from about nucleotide 982 to about nucleotide 1011 is useful, for example, for designing oligonucleotides or as a hybridization probe. Northern analysis shows the expression of this sequence in various libraries, at least 63% of which are immortalized or cancerous and at least 26% of which involve immune response. Of particular note is the expression of GAPIP in reproductive, gastrointestinal, nervous, and fetal tissues.

The invention also encompasses GAPIP variants. A preferred GAPIP variant is one which has at least about 80%, more preferably at least about 90%, and most preferably at least about 95% amino acid sequence identity to the GAPIP amino acid sequence, and which contains at least one functional or structural characteristic of GAPIP.

The invention also encompasses polynucleotides which encode GAPIP. In a particular embodiment, the invention encompasses a polynucleotide sequence comprising the sequence of SEQ ID NO:2, which encodes an GAPIP.

The invention also encompasses a variant of a polynucleotide sequence encoding GAPIP. In particular, such a variant polynucleotide sequence will have at least about 80%, more preferably at least about 90%, and most preferably at least about 95% polynucleotide sequence identity to the polynucleotide sequence encoding GAPIP. A particular aspect of the invention encompasses a variant of SEQ ID NO:2 which has at least about 80%, more preferably at least about 90%, and most preferably at least about 95% polynucleotide sequence identity to SEQ ID NO:2. Any one of the polynucleotide variants described above can encode an amino acid sequence which contains at least one functional or structural characteristic of GAPIP.

It will be appreciated by those skilled in the art that as a result of the degeneracy of the genetic code, a multitude of polynucleotide sequences encoding GAPIP, some bearing minimal similarity to the polynucleotide sequences of any known and naturally occurring gene, may be produced. Thus, the invention contemplates each and every possible variation of polynucleotide sequence that could be made by selecting combinations based on possible codon choices. These combinations are made in accordance with the standard triplet genetic code as applied to the polynucleotide sequence of naturally occurring GAPIP, and all such variations are to be considered as

being specifically disclosed.

Although nucleotide sequences which encode GAPIP and its variants are preferably capable of hybridizing to the nucleotide sequence of the naturally occurring GAPIP under appropriately selected conditions of stringency, it may be advantageous to produce nucleotide sequences encoding 5 GAPIP or its derivatives possessing a substantially different codon usage, e.g., inclusion of non-naturally occurring codons. Codons may be selected to increase the rate at which expression of the peptide occurs in a particular prokaryotic or eukaryotic host in accordance with the frequency with which particular codons are utilized by the host. Other reasons for substantially altering the nucleotide sequence encoding GAPIP and its derivatives without altering the encoded amino acid 10 sequences include the production of RNA transcripts having more desirable properties, such as a greater half-life, than transcripts produced from the naturally occurring sequence.

The invention also encompasses production of DNA sequences which encode GAPIP and GAPIP derivatives, or fragments thereof, entirely by synthetic chemistry. After production, the synthetic sequence may be inserted into any of the many available expression vectors and cell systems using reagents well known in the art. Moreover, synthetic chemistry may be used to introduce mutations into a sequence encoding GAPIP or any fragment thereof.

Also encompassed by the invention are polynucleotide sequences that are capable of hybridizing to the claimed polynucleotide sequences, and, in particular, to those shown in SEQ ID NO:2, or a fragment of SEQ ID NO:2, under various conditions of stringency. (See, e.g., Wahl, G.M. and S.L. Berger (1987) Methods Enzymol. 152:399-407; Kimmel, A.R. (1987) Methods Enzymol. 20 152:507-511.)

Methods for DNA sequencing are well known and generally available in the art and may be used to practice any of the embodiments of the invention. The methods may employ such enzymes as the Klenow fragment of DNA polymerase I, SEQUENASE, Taq DNA polymerase and thermostable 25 T7 DNA polymerase (Amersham Pharmacia Biotech (APB), Piscataway NJ), or combinations of polymerases and proofreading exonucleases such as those found in the ELONGASE amplification system (Life Technologies, Gaithersburg MD). Preferably, sequence preparation is automated with machines such as the MICROLAB 2200 system (Hamilton, Reno NV) and the DNA ENGINE thermal cycler (MJ Research, Watertown MA). Machines commonly used for sequencing include the 30 ABI PRISM 3700, 377 or 373 DNA sequencing systems (Applied Biosystems), the MEGABACE 1000 DNA sequencing system (APB), and the like.

The nucleic acid sequences encoding GAPIP may be extended utilizing a partial nucleotide sequence and employing various PCR-based methods known in the art to detect upstream sequences,

such as promoters and regulatory elements. For example, one method which may be employed, restriction-site PCR, uses universal and nested primers to amplify unknown sequence from genomic DNA within a cloning vector. (See, e.g., Sarkar, G. (1993) PCR Methods Applic. 2:318-322.)

Another method, inverse PCR, uses primers that extend in divergent directions to amplify unknown sequence from a circularized template. The template is derived from restriction fragments comprising a known genomic locus and surrounding sequences. (See, e.g., Triglia, T. et al. (1988) Nucleic Acids Res. 16:8186.) A third method, capture PCR, involves PCR amplification of DNA fragments adjacent to known sequences in human and yeast artificial chromosome DNA. (See, e.g., Lagerstrom, M. et al. (1991) PCR Methods Applic. 1:111-119.) In this method, multiple restriction enzyme digestions and ligations may be used to insert an engineered double-stranded sequence into a region of unknown sequence before performing PCR. Other methods which may be used to retrieve unknown sequences are known in the art. (See, e.g., Parker, J.D. et al. (1991) Nucleic Acids Res. 19:3055-306).

Additionally, one may use PCR, nested primers, and PROMOTERFINDER libraries to walk genomic DNA (Clontech, Palo Alto, CA). This procedure avoids the need to screen libraries and is useful in finding intron/exon junctions. For all PCR-based methods, primers may be designed using commercially available software, such as OLIGO 4.06 Primer Analysis software (National Biosciences Inc., Plymouth, MN) or another appropriate program, to be about 22 to 30 nucleotides in length, to have a GC content of about 50% or more, and to anneal to the template at temperatures of about 68°C to 72°C.

When screening for full-length cDNAs, it is preferable to use libraries that have been size-selected to include larger cDNAs. In addition, random-primed libraries, which often include sequences containing the 5' regions of genes, are preferable for situations in which an oligo d(T) library does not yield a full-length cDNA. Genomic libraries may be useful for extension of sequence into 5' non-transcribed regulatory regions.

Capillary electrophoresis systems which are commercially available may be used to analyze the size or confirm the nucleotide sequence of sequencing or PCR products. In particular, capillary sequencing may employ flowable polymers for electrophoretic separation, four different nucleotide-specific, laser-stimulated fluorescent dyes, and a charge coupled device camera for detection of the emitted wavelengths. Output/light intensity may be converted to electrical signal using appropriate software (e.g., GENOTYPER and SEQUENCE NAVIGATOR, Applied Biosystems), and the entire process from loading of samples to computer analysis and electronic data display may be computer controlled. Capillary electrophoresis is especially preferable for sequencing small DNA fragments which may be present in limited amounts in a particular sample.

In another embodiment of the invention, polynucleotide sequences or fragments thereof which encode GAPIP may be cloned in recombinant DNA molecules that direct expression of GAPIP, or fragments or functional equivalents thereof, in appropriate host cells. Due to the inherent degeneracy of the genetic code, other DNA sequences which encode substantially the same or a functionally equivalent amino acid sequence may be produced and used to express GAPIP.

The nucleotide sequences of the present invention can be engineered using methods generally known in the art in order to alter GAPIP-encoding sequences for a variety of purposes including, but not limited to, modification of the cloning, processing, and/or expression of the gene product. DNA shuffling by random fragmentation and PCR reassembly of gene fragments and synthetic oligonucleotides may be used to engineer the nucleotide sequences. For example, oligonucleotide-mediated site-directed mutagenesis may be used to introduce mutations that create new restriction sites, alter glycosylation patterns, change codon preference, produce splice variants, and so forth.

In another embodiment, sequences encoding GAPIP may be synthesized, in whole or in part, using chemical methods well known in the art. (See, e.g., Caruthers, M.H. et al. (1980) Nucl. Acids Symp. Ser. 7:215-223, and Horn, T. et al. (1980) Nucl. Acids Symp. Ser. 7:225-232.) Alternatively, GAPIP itself or a fragment thereof may be synthesized using chemical methods. For example, peptide synthesis can be performed using various solid-phase techniques. (See, e.g., Roberge, J.Y. et al. (1995) Science 269:202-204.) Automated synthesis may be achieved using the ABI 431A Peptide Synthesizer (Perkin Elmer). Additionally, the amino acid sequence of GAPIP, or any part thereof, may be altered during direct synthesis and/or combined with sequences from other proteins, or any part thereof, to produce a variant polypeptide.

The peptide may be substantially purified by preparative high performance liquid chromatography. (See, e.g., Chiez, R.M. and F.Z. Regnier (1990) Methods Enzymol. 182:392-421.) The composition of the synthetic peptides may be confirmed by amino acid analysis or by sequencing. (See, e.g., Creighton, T. (1984) Proteins, Structures and Molecular Properties, WH Freeman and Co., New York, NY.)

In order to express a biologically active GAPIP, the nucleotide sequences encoding GAPIP or derivatives thereof may be inserted into an appropriate expression vector, i.e., a vector which contains the necessary elements for transcriptional and translational control of the inserted coding sequence in a suitable host. These elements include regulatory sequences, such as enhancers, constitutive and inducible promoters, and 5' and 3' untranslated regions in the vector and in polynucleotide sequences encoding GAPIP. Such elements may vary in their strength and specificity. Specific initiation signals may also be used to achieve more efficient translation of sequences encoding GAPIP. Such signals

include the ATG initiation codon and adjacent sequences, e.g. the Kozak sequence. In cases where sequences encoding GAPIP and its initiation codon and upstream regulatory sequences are inserted into the appropriate expression vector, no additional transcriptional or translational control signals may be needed. However, in cases where only coding sequence, or a fragment thereof, is inserted, exogenous translational control signals including an in-frame ATG initiation codon should be provided by the vector. Exogenous translational elements and initiation codons may be of various origins, both natural and synthetic. The efficiency of expression may be enhanced by the inclusion of enhancers appropriate for the particular host cell system used. (See, e.g., Scharf, D. et al. (1994) Results Probl. Cell Differ. 20:125-162.)

Methods which are well known to those skilled in the art may be used to construct expression vectors containing sequences encoding GAPIP and appropriate transcriptional and translational control elements. These methods include in vitro recombinant DNA techniques, synthetic techniques, and in vivo genetic recombination. (See, e.g., Sambrook, J. et al. (1989) Molecular Cloning, A Laboratory Manual, Cold Spring Harbor Press, Plainview, NY, ch. 4, 8, and 16-17; and Ausubel, F.M. et al. (1995, and periodic supplements) Current Protocols in Molecular Biology, John Wiley & Sons, New York, NY, ch. 9, 13, and 16.)

A variety of expression vector/host systems may be utilized to contain and express sequences encoding GAPIP. These include, but are not limited to, microorganisms such as bacteria transformed with recombinant bacteriophage, plasmid, or cosmid DNA expression vectors; yeast transformed with yeast expression vectors; insect cell systems infected with viral expression vectors (e.g., baculovirus); plant cell systems transformed with viral expression vectors (e.g., cauliflower mosaic virus (CaMV) or tobacco mosaic virus (TMV)) or with bacterial expression vectors (e.g., Ti or pBR322 plasmids); or animal cell systems. The invention is not limited by the host cell employed.

In bacterial systems, a number of cloning and expression vectors may be selected depending upon the use intended for polynucleotide sequences encoding GAPIP. For example, routine cloning, subcloning, and propagation of polynucleotide sequences encoding GAPIP can be achieved using a multifunctional E. coli vector such as BLUESCRIPT phagemid (Stratagene, La Jolla, CA) or PSPORT1 plasmid (Life Technologies). Ligation of sequences encoding GAPIP into the vector's multiple cloning site disrupts the *lacZ* gene, allowing a colorimetric screening procedure for identification of transformed bacteria containing recombinant molecules. In addition, these vectors may be useful for in vitro transcription, dideoxy sequencing, single strand rescue with helper phage, and creation of nested deletions in the cloned sequence. (See, e.g., Van Heeke, G. and S.M. Schuster (1989) J. Biol. Chem. 264:5503-5509.) When large quantities of GAPIP are needed, e.g. for the

production of antibodies, vectors which direct high level expression of GAPIP may be used. For example, vectors containing the strong, inducible T5 or T7 bacteriophage promoter may be used.

Yeast expression systems may be used for production of GAPIP. A number of vectors containing constitutive or inducible promoters, such as alpha factor, alcohol oxidase, and PGH, may 5 be used in the yeast Saccharomyces cerevisiae or Pichia pastoris. In addition, such vectors direct either the secretion or intracellular retention of expressed proteins and enable integration of foreign sequences into the host genome for stable propagation. (See, e.g., Ausubel, supra; and Grant et al. (1987) Methods Enzymol. 153:516-54; Scorer, C. A. et al. (1994) Bio/Technology 12:181-184.)

Plant systems may also be used for expression of GAPIP. Transcription of sequences 10 encoding GAPIP may be driven viral promoters, e.g., the 35S and 19S promoters of CaMV used alone or in combination with the omega leader sequence from TMV. (Takamatsu, N. (1987) EMBO J. 6:307-311.) Alternatively, plant promoters such as the small subunit of RUBISCO or heat shock promoters may be used. (See, e.g., Coruzzi, G. et al. (1984) EMBO J. 3:1671-1680; Broglie, R. et al. (1984) Science 224:838-843; and Winter, J. et al. (1991) Results Probl. Cell Differ. 17:85-105.) These constructs can be introduced into plant cells by direct DNA transformation or pathogen-mediated transfection. (See, e.g., Hobbs, S. or Murry, L.E. in McGraw Hill Yearbook of Science and Technology (1992) McGraw Hill, New York, NY; pp. 191-196.)

In mammalian cells, a number of viral-based expression systems may be utilized. In cases where an adenovirus is used as an expression vector, sequences encoding GAPIP may be ligated into an adenovirus transcription/translation complex consisting of the late promoter and tripartite leader sequence. Insertion in a non-essential E1 or E3 region of the viral genome may be used to obtain infective virus which expresses GAPIP in host cells. (See, e.g., Logan, J. and T. Shenk (1984) Proc. Natl. Acad. Sci. 81:3655-3659.) In addition, transcription enhancers, such as the Rous sarcoma virus (RSV) enhancer, may be used to increase expression in mammalian host cells. SV40 or EBV-based 25 vectors may also be used for high-level protein expression.

Human artificial chromosomes (HACs) may also be employed to deliver larger fragments of DNA than can be contained in and expressed from a plasmid. HACs of about 6 kb to 10 Mb are constructed and delivered via conventional delivery methods (liposomes, polycationic amino polymers, or vesicles) for therapeutic purposes.

For long term production of recombinant proteins in mammalian systems, stable expression of 30 GAPIP in cell lines is preferred. For example, sequences encoding GAPIP can be transformed into cell lines using expression vectors which may contain viral origins of replication and/or endogenous expression elements and a selectable marker gene on the same or on a separate vector. Following the

introduction of the vector, cells may be allowed to grow for about 1 to 2 days in enriched media before being switched to selective media. The purpose of the selectable marker is to confer resistance to a selective agent, and its presence allows growth and recovery of cells which successfully express the introduced sequences. Resistant clones of stably transformed cells may be propagated using tissue culture techniques appropriate to the cell type.

Any number of selection systems may be used to recover transformed cell lines. These include, but are not limited to, the herpes simplex virus thymidine kinase and adenine phosphoribosyltransferase genes, for use in *tk*<sup>-</sup> or *apr*<sup>-</sup> cells, respectively. (See, e.g., Wigler, M. et al. (1977) Cell 11:223-232; and Lowy, I. et al. (1980) Cell 22:817-823.) Also, antimetabolite, antibiotic, or herbicide resistance can be used as the basis for selection. For example, *dhfr* confers resistance to methotrexate; *neo* confers resistance to the aminoglycosides neomycin and G-418; and *als* or *pat* confer resistance to chlorsulfuron and phosphinotricin acetyltransferase, respectively. (See, e.g., Wigler, M. et al. (1980) Proc. Natl. Acad. Sci. 77:3567-3570; Colbere-Garapin, F. et al (1981) J. Mol. Biol. 150:1-14; and Murry, supra.) Additional selectable genes have been described, e.g., *trpB* and *hisD*, which alter cellular requirements for metabolites. (See, e.g., Hartman, S.C. and R.C. Mulligan (1988) Proc. Natl. Acad. Sci. 85:8047-8051.) Visible markers, e.g., anthocyanins, green fluorescent proteins (GFP) (Clontech, Palo Alto, CA),  $\beta$  glucuronidase and its substrate  $\beta$ -D-glucuronoside, or luciferase and its substrate luciferin may be used. These markers can be used not only to identify transformants, but also to quantify the amount of transient or stable protein expression attributable to a specific vector system. (See, e.g., Rhodes, C.A. et al. (1995) Methods Mol. Biol. 55:121-131.)

Although the presence/absence of marker gene expression suggests that the gene of interest is also present, the presence and expression of the gene may need to be confirmed. For example, if the sequence encoding GAPIP is inserted within a marker gene sequence, transformed cells containing sequences encoding GAPIP can be identified by the absence of marker gene function. Alternatively, a marker gene can be placed in tandem with a sequence encoding GAPIP under the control of a single promoter. Expression of the marker gene in response to induction or selection usually indicates expression of the tandem gene as well.

In general, host cells that contain the nucleic acid sequence encoding GAPIP and that express GAPIP may be identified by a variety of procedures known to those of skill in the art. These procedures include, but are not limited to, DNA-DNA or DNA-RNA hybridizations, PCR amplification, and protein bioassay or immunoassay techniques which include membrane, solution, or chip based technologies for the detection and/or quantification of nucleic acid or protein sequences.

Immunological methods for detecting and measuring the expression of GAPIP using either

specific polyclonal or monoclonal antibodies are known in the art. Examples of such techniques include enzyme-linked immunosorbent assays (ELISAs), radioimmunoassays (RIAs), and fluorescence activated cell sorting (FACS). A two-site, monoclonal-based immunoassay utilizing monoclonal antibodies reactive to two non-interfering epitopes on GAPIP is preferred, but a competitive binding assay may be employed. These and other assays are well known in the art. (See, e.g., Hampton, R. et al. (1990) Serological Methods, a Laboratory Manual, APS Press, St Paul, MN, Section IV; Coligan, J. E. et al. (1997 and periodic supplements) Current Protocols in Immunology, Greene Pub. Associates and Wiley-Interscience, New York, NY; and Maddox, D.E. et al. (1983) *J. Exp. Med.* 158:1211-1216).

A wide variety of labels and conjugation techniques are known by those skilled in the art and may be used in various nucleic acid and amino acid assays. Means for producing labeled hybridization or PCR probes for detecting sequences related to polynucleotides encoding GAPIP include oligolabeling, nick translation, end-labeling, or PCR amplification using a labeled nucleotide. Alternatively, the sequences encoding GAPIP, or any fragments thereof, may be cloned into a vector for the production of an mRNA probe. Such vectors are known in the art, are commercially available, and may be used to synthesize RNA probes in vitro by addition of an appropriate RNA polymerase such as T7, T3, or SP6 and labeled nucleotides. These procedures may be conducted using a variety of commercially available kits, such as those provided by Promega (Madison WI) or Amersham Pharmacia Biotech. Suitable reporter molecules or labels which may be used for ease of detection include radionuclides, enzymes, fluorescent, chemiluminescent, or chromogenic agents, as well as substrates, cofactors, inhibitors, magnetic particles, and the like.

Host cells transformed with nucleotide sequences encoding GAPIP may be cultured under conditions suitable for the expression and recovery of the protein from cell culture. The protein produced by a transformed cell may be secreted or retained intracellularly depending on the sequence and/or the vector used. As will be understood by those of skill in the art, expression vectors containing polynucleotides which encode GAPIP may be designed to contain signal sequences which direct secretion of GAPIP through a prokaryotic or eukaryotic cell membrane.

In addition, a host cell strain may be chosen for its ability to modulate expression of the inserted sequences or to process the expressed protein in the desired fashion. Such modifications of the polypeptide include, but are not limited to, acetylation, carboxylation, glycosylation, phosphorylation, lipidation, and acylation. Post-translational processing which cleaves a "prepro" form of the protein may also be used to specify protein targeting, folding, and/or activity. Different host cells which have specific cellular machinery and characteristic mechanisms for post-translational activities (e.g., CHO, HeLa, MDCK, HEK293, and WI38), are available from the American Type

Culture Collection (ATCC, Bethesda, MD) and may be chosen to ensure the correct modification and processing of the foreign protein.

In another embodiment of the invention, natural, modified, or recombinant nucleic acid sequences encoding GAPIP may be ligated to a heterologous sequence resulting in translation of a fusion protein in any of the aforementioned host systems. For example, a chimeric GAPIP protein containing a heterologous moiety that can be recognized by a commercially available antibody may facilitate the screening of peptide libraries for inhibitors of GAPIP activity. Heterologous protein and peptide moieties may also facilitate purification of fusion proteins using commercially available affinity matrices. Such moieties include, but are not limited to, glutathione S-transferase (GST), maltose binding protein (MBP), thioredoxin (Trx), calmodulin binding peptide (CBP), 6-His, FLAG, *c-myc*, and hemagglutinin (HA). GST, MBP, Trx, CBP, and 6-His enable purification of their cognate fusion proteins on immobilized glutathione, maltose, phenylarsine oxide, calmodulin, and metal-chelate resins, respectively. FLAG, *c-myc*, and hemagglutinin (HA) enable immunoaffinity purification of fusion proteins using commercially available monoclonal and polyclonal antibodies that specifically recognize these epitope tags. A fusion protein may also be engineered to contain a proteolytic cleavage site located between the GAPIP encoding sequence and the heterologous protein sequence, so that GAPIP may be cleaved away from the heterologous moiety following purification. Methods for fusion protein expression and purification are discussed in Ausubel, F. M. et al. (1995 and periodic supplements) Current Protocols in Molecular Biology, John Wiley & Sons, New York, NY, ch 10. A variety of commercially available kits may also be used to facilitate expression and purification of fusion proteins.

In a further embodiment of the invention, synthesis of radiolabeled GAPIP may be achieved in vitro using the TNT rabbit reticulocyte lysate or wheat germ extract systems (Promega). These systems couple transcription and translation of protein-coding sequences operably associated with the T7, T3, or SP6 promoters. Translation takes place in the presence of a radiolabeled amino acid precursor, preferably <sup>35</sup>S-methionine.

Fragments of GAPIP may be produced not only by recombinant production, but also by direct peptide synthesis using solid-phase techniques. (See, e.g., Creighton, supra pp. 55-60.) Protein synthesis may be performed by manual techniques or by automation. Automated synthesis may be achieved, for example, using the Applied Biosystems 431A peptide synthesizer. Various fragments of GAPIP may be synthesized separately and then combined to produce the full length molecule.

**THERAPEUTICS**

Chemical and structural similarity exists among GAPIP and human pre-inter- $\alpha$ -trypsin inhibitor (GI 33985; SEQ ID NO:3), human pre-inter- $\alpha$ -trypsin inhibitor heavy chain H1 (GI 33989; SEQ ID NO:4), and human pre-inter- $\alpha$ -trypsin inhibitor heavy chain H3 (GI 288563; SEQ ID NO:5).  
5 In addition, GAPIP is expressed in cancer, immune, reproductive, gastrointestinal, nervous, and fetal tissues. Therefore, GAPIP appears to play a role in reproductive, developmental, neoplastic, and immunological disorders.

Therefore, in one embodiment, a pharmaceutical composition comprising a substantially purified GAPIP in conjunction with a suitable pharmaceutical carrier may be administered to a subject 10 to treat or prevent a reproductive disorder. Such reproductive disorders can include, but are not limited to, disorders of prolactin production; infertility, including tubal disease, ovulatory defects, and endometriosis; disruptions of the estrous cycle, disruptions of the menstrual cycle, polycystic ovary syndrome, ovarian hyperstimulation syndrome, endometrial and ovarian tumors, uterine fibroids, autoimmune disorders, ectopic pregnancies, and teratogenesis; cancer of the breast, fibrocystic breast disease, and galactorrhea; disruptions of spermatogenesis, abnormal sperm physiology, cancer of the testis, cancer of the prostate, benign prostatic hyperplasia, prostatitis, Peyronie's disease, carcinoma of the male breast, and gynecomastia.  
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In another embodiment, a vector capable of expressing GAPIP or a fragment or derivative thereof may be administered to a subject to treat or prevent a reproductive disorder including, but not limited to, those described above.

In a further embodiment, GAPIP or a fragment or derivative thereof may be administered to a subject to treat or prevent a reproductive disorder including, but not limited to, those provided above.

In still another embodiment, an agonist which modulates the activity of GAPIP may be administered to a subject to treat or prevent a reproductive disorder including, but not limited to, those listed above.  
25

In one embodiment, a pharmaceutical composition comprising a substantially purified GAPIP in conjunction with a suitable pharmaceutical carrier may be administered to a subject to treat or prevent a developmental disorder. The term "developmental disorder" refers to any disorder associated with growth and differentiation, embryogenesis, and morphogenesis involving any tissue, 30 organ, or system of a subject (such as the brain, adrenal gland, kidney, skeletal or reproductive system). Such developmental disorders can include, but are not limited to, renal tubular acidosis, anemia, Cushing's syndrome, achondroplastic dwarfism, Duchenne and Becker muscular dystrophy, epilepsy, gonadal dysgenesis, WAGR syndrome (Wilms' tumor, aniridia, genitourinary abnormalities,

and mental retardation), Smith-Magenis syndrome, myelodysplastic syndrome, hereditary mucoepithelial dysplasia, hereditary keratodermas, hereditary neuropathies such as Charcot-Marie-Tooth disease and neurofibromatosis, hypothyroidism, hydrocephalus, seizure disorders such as Sydenham's chorea and cerebral palsy, spinal bifida, congenital glaucoma, cataract, and sensorineural hearing loss.

5 In another embodiment, a vector capable of expressing GAPIP or a fragment or derivative thereof may be administered to a subject to treat or prevent a developmental disorder including, but not limited to, those described above.

10 In a further embodiment, GAPIP or a fragment or derivative thereof may be administered to a subject to treat or prevent a developmental disorder including, but not limited to, those provided above.

In still another embodiment, an agonist which modulates the activity of GAPIP may be administered to a subject to treat or prevent a developmental disorder including, but not limited to, those listed above.

15 In one embodiment, an antagonist of GAPIP may be administered to a subject to treat or prevent a neoplastic disorder. Such neoplastic disorders may include, but are not limited to, adenocarcinoma, leukemia, lymphoma, melanoma, myeloma, sarcoma, teratocarcinoma, and, in particular, cancers of the adrenal gland, bladder, bone, bone marrow, brain, breast, cervix, gall bladder, ganglia, gastrointestinal tract, heart, kidney, liver, lung, muscle, ovary, pancreas, parathyroid, penis, prostate, salivary glands, skin, spleen, testis, thymus, thyroid, and uterus. In one aspect, an antibody which specifically binds GAPIP may be used directly as an antagonist or indirectly as a targeting or delivery mechanism for bringing a pharmaceutical agent to cells or tissue which express GAPIP.

20 In an additional embodiment, a vector expressing the complement of the polynucleotide encoding GAPIP may be administered to a subject to treat or prevent a neoplastic disorder including, but not limited to, those described above.

25 In one embodiment, an antagonist of GAPIP may be administered to a subject to treat or prevent an immunological disorder. Such immunological disorders may include, but are not limited to, acquired immunodeficiency syndrome (AIDS), Addison's disease, adult respiratory distress syndrome, allergies, ankylosing spondylitis, amyloidosis, anemia, asthma, atherosclerosis, autoimmune hemolytic anemia, autoimmune thyroiditis, bronchitis, cholecystitis, contact dermatitis, Crohn's disease, atopic dermatitis, dermatomyositis, diabetes mellitus, emphysema, episodic lymphopenia with lymphocytotoxins, erythroblastosis fetalis, erythema nodosum, atrophic gastritis,

glomerulonephritis, Goodpasture's syndrome, gout, Graves' disease, Hashimoto's thyroiditis, hypereosinophilia, irritable bowel syndrome, multiple sclerosis, myasthenia gravis, myocardial or pericardial inflammation, osteoarthritis, osteoporosis, pancreatitis, polymyositis, psoriasis, Reiter's syndrome, rheumatoid arthritis, scleroderma, Sjögren's syndrome, systemic anaphylaxis, systemic lupus erythematosus, systemic sclerosis, thrombocytopenic purpura, ulcerative colitis, uveitis, Werner syndrome, complications of cancer, hemodialysis, and extracorporeal circulation, viral, bacterial, fungal, parasitic, protozoal, and helminthic infections, and trauma; and arteriosclerosis, bursitis, cirrhosis, hepatitis, mixed connective tissue disease (MCTD), myelofibrosis, paroxysmal nocturnal hemoglobinuria, polycythemia vera, and primary thrombocythemia. In one aspect, an antibody which specifically binds GAPIP may be used directly as an antagonist or indirectly as a targeting or delivery mechanism for bringing a pharmaceutical agent to cells or tissue which express GAPIP.

In an additional embodiment, a vector expressing the complement of the polynucleotide encoding GAPIP may be administered to a subject to treat or prevent an immunological disorder including, but not limited to, those described above.

In other embodiments, any of the proteins, antagonists, antibodies, agonists, complementary sequences, or vectors of the invention may be administered in combination with other appropriate therapeutic agents. Selection of the appropriate agents for use in combination therapy may be made by one of ordinary skill in the art, according to conventional pharmaceutical principles. The combination of therapeutic agents may act synergistically to effect the treatment or prevention of the various disorders described above. Using this approach, one may be able to achieve therapeutic efficacy with lower dosages of each agent, thus reducing the potential for adverse side effects.

An antagonist of GAPIP may be produced using methods which are generally known in the art. In particular, purified GAPIP may be used to produce antibodies or to screen libraries of pharmaceutical agents to identify those which specifically bind GAPIP. Antibodies to GAPIP may also be generated using methods that are well known in the art. Such antibodies may include, but are not limited to, polyclonal, monoclonal, chimeric, and single chain antibodies, Fab fragments, and fragments produced by a Fab expression library. Neutralizing antibodies (i.e., those which inhibit dimer formation) are especially preferred for therapeutic use.

For the production of antibodies, various hosts including goats, rabbits, rats, mice, humans, and others may be immunized by injection with GAPIP or with any fragment or oligopeptide thereof which has immunogenic properties. Depending on the host species, various adjuvants may be used to increase immunological response. Such adjuvants include, but are not limited to, Freund's, mineral gels such as aluminum hydroxide, and surface active substances such as lysolecithin, pluronic polyols,

polyanions, peptides, oil emulsions, KLH, and dinitrophenol. Among adjuvants used in humans, BCG (bacilli Calmette-Guerin) and Corynebacterium parvum are especially preferable.

It is preferred that the oligopeptides, peptides, or fragments used to induce antibodies to GAPIP have an amino acid sequence consisting of at least about 5 amino acids, and, more preferably, 5 of at least about 10 amino acids. It is also preferable that these oligopeptides, peptides, or fragments are identical to a portion of the amino acid sequence of the natural protein and contain the entire amino acid sequence of a small, naturally occurring molecule. Short stretches of GAPIP amino acids may be fused with those of another protein, such as KLH, and antibodies to the chimeric molecule may be produced.

10 Monoclonal antibodies to GAPIP may be prepared using any technique which provides for the production of antibody molecules by continuous cell lines in culture. These include, but are not limited to, the hybridoma technique, the human B-cell hybridoma technique, and the EBV-hybridoma technique. (See, e.g., Kohler, G. et al. (1975) *Nature* 256:495-497; Kozbor, D. et al. (1985) *J. Immunol. Methods* 81:31-42; Cote, R.J. et al. (1983) *Proc. Natl. Acad. Sci.* 80:2026-2030; and Cole, S.P. et al. (1984) *Mol. Cell Biol.* 62:109-120.)

15 In addition, techniques developed for the production of "chimeric antibodies," such as the splicing of mouse antibody genes to human antibody genes to obtain a molecule with appropriate antigen specificity and biological activity, can be used. (See, e.g., Morrison, S.L. et al. (1984) *Proc. Natl. Acad. Sci.* 81:6851-6855; Neuberger, M.S. et al. (1984) *Nature* 312:604-608; and Takeda, S. et al. (1985) *Nature* 314:452-454.) Alternatively, techniques described for the production of single chain antibodies may be adapted, using methods known in the art, to produce GAPIP-specific single chain antibodies. Antibodies with related specificity, but of distinct idiotypic composition, may be generated by chain shuffling from random combinatorial immunoglobulin libraries. (See, e.g., Burton D.R. (1991) *Proc. Natl. Acad. Sci.* 88:10134-10137.)

20 Antibodies may also be produced by inducing in vivo production in the lymphocyte population or by screening immunoglobulin libraries or panels of highly specific binding reagents as disclosed in the literature. (See, e.g., Orlandi, R. et al. (1989) *Proc. Natl. Acad. Sci.* 86: 3833-3837; and Winter, G. et al. (1991) *Nature* 349:293-299.)

25 Antibody fragments which contain specific binding sites for GAPIP may also be generated. For example, such fragments include, but are not limited to, F(ab')2 fragments produced by pepsin digestion of the antibody molecule and Fab fragments generated by reducing the disulfide bridges of the F(ab')2 fragments. Alternatively, Fab expression libraries may be constructed to allow rapid and easy identification of monoclonal Fab fragments with the desired specificity. (See, e.g., Huse, W.D. et

al. (1989) Science 246:1275-1281.)

Various immunoassays may be used for screening to identify antibodies having the desired specificity. Numerous protocols for competitive binding or immunoradiometric assays using either polyclonal or monoclonal antibodies with established specificities are well known in the art. Such immunoassays typically involve the measurement of complex formation between GAPIP and its specific antibody. A two-site, monoclonal-based immunoassay utilizing monoclonal antibodies reactive to two non-interfering GAPIP epitopes is preferred, but a competitive binding assay may also be employed. (Maddox, supra.)

In another embodiment of the invention, the polynucleotides encoding GAPIP, or any fragment or complement thereof, may be used for therapeutic purposes. In one aspect, the complement of the polynucleotide encoding GAPIP may be used in situations in which it would be desirable to block the transcription of the mRNA. In particular, cells may be transformed with sequences complementary to polynucleotides encoding GAPIP. Thus, complementary molecules or fragments may be used to modulate GAPIP activity, or to achieve regulation of gene function. Such technology is now well known in the art, and sense or antisense oligonucleotides or larger fragments can be designed from various locations along the coding or control regions of sequences encoding GAPIP.

Expression vectors derived from retroviruses, adenoviruses, or herpes or vaccinia viruses, or from various bacterial plasmids, may be used for delivery of nucleotide sequences to the targeted organ, tissue, or cell population. Methods which are well known to those skilled in the art can be used to construct vectors to express nucleic acid sequences complementary to the polynucleotides encoding GAPIP. (See, e.g., Sambrook, supra; and Ausubel, supra.)

Genes encoding GAPIP can be turned off by transforming a cell or tissue with expression vectors which express high levels of a polynucleotide, or fragment thereof, encoding GAPIP. Such constructs may be used to introduce untranslatable sense or antisense sequences into a cell. Even in the absence of integration into the DNA, such vectors may continue to transcribe RNA molecules until they are disabled by endogenous nucleases. Transient expression may last for a month or more with a non-replicating vector, and may last even longer if appropriate replication elements are part of the vector system.

As mentioned above, modifications of gene expression can be obtained by designing complementary sequences or antisense molecules (DNA, RNA, or PNA) to the control, 5', or regulatory regions of the gene encoding GAPIP. Oligonucleotides derived from the transcription initiation site, e.g., between about positions -10 and +10 from the start site, are preferred. Similarly,

inhibition can be achieved using triple helix base-pairing methodology. Triple helix pairing is useful because it causes inhibition of the ability of the double helix to open sufficiently for the binding of polymerases, transcription factors, or regulatory molecules. Recent therapeutic advances using triplex DNA have been described in the literature. (See, e.g., Gee, J.E. et al. (1994) in Huber, B.E. and B.I. Carr, Molecular and Immunologic Approaches, Futura Publishing Co., Mt. Kisco, NY, pp. 163-177.) A complementary sequence or antisense molecule may also be designed to block translation of mRNA by preventing the transcript from binding to ribosomes.

Ribozymes, enzymatic RNA molecules, may also be used to catalyze the specific cleavage of RNA. The mechanism of ribozyme action involves sequence-specific hybridization of the ribozyme molecule to complementary target RNA, followed by endonucleolytic cleavage. For example, engineered hammerhead motif ribozyme molecules may specifically and efficiently catalyze endonucleolytic cleavage of sequences encoding GAPIP.

Specific ribozyme cleavage sites within any potential RNA target are initially identified by scanning the target molecule for ribozyme cleavage sites, including the following sequences: GUA, GUU, and GUC. Once identified, short RNA sequences of between 15 and 20 ribonucleotides, corresponding to the region of the target gene containing the cleavage site, may be evaluated for secondary structural features which may render the oligonucleotide inoperable. The suitability of candidate targets may also be evaluated by testing accessibility to hybridization with complementary oligonucleotides using ribonuclease protection assays.

Complementary ribonucleic acid molecules and ribozymes of the invention may be prepared by any method known in the art for the synthesis of nucleic acid molecules. These include techniques for chemically synthesizing oligonucleotides such as solid phase phosphoramidite chemical synthesis. Alternatively, RNA molecules may be generated by in vitro and in vivo transcription of DNA sequences encoding GAPIP. Such DNA sequences may be incorporated into a wide variety of vectors with suitable RNA polymerase promoters such as T7 or SP6. Alternatively, these cDNA constructs that synthesize complementary RNA, constitutively or inducibly, can be introduced into cell lines, cells, or tissues.

RNA molecules may be modified to increase intracellular stability and half-life. Possible modifications include, but are not limited to, the addition of flanking sequences at the 5' and/or 3' ends of the molecule, or the use of phosphorothioate or 2' O-methyl rather than phosphodiesterase linkages within the backbone of the molecule. This concept is inherent in the production of PNAs and can be extended in all of these molecules by the inclusion of nontraditional bases such as inosine, queosine, and wybutosine, as well as

acetyl-, methyl-, thio-, and similarly modified forms of adenine, cytidine, guanine, thymine, and uridine which are not as easily recognized by endogenous endonucleases.

Many methods for introducing vectors into cells or tissues are available and equally suitable for use in vivo, in vitro, and ex vivo. For ex vivo therapy, vectors may be introduced into stem cells taken from the patient and clonally propagated for autologous transplant back into that same patient. Delivery by transfection, by liposome injections, or by polycationic amino polymers may be achieved using methods which are well known in the art. (See, e.g., Goldman, C.K. et al. (1997) *Nature Biotechnology* 15:462-466.)

Any of the therapeutic methods described above may be applied to any subject in need of such therapy, including, for example, mammals such as dogs, cats, cows, horses, rabbits, monkeys, and most preferably, humans.

An additional embodiment of the invention relates to the administration of a pharmaceutical or sterile composition, in conjunction with a pharmaceutically acceptable carrier, for any of the therapeutic effects discussed above. Such pharmaceutical compositions may consist of GAPIP, antibodies to GAPIP, and mimetics, agonists, antagonists, or inhibitors of GAPIP. The compositions may be administered alone or in combination with at least one other agent, such as a stabilizing compound, which may be administered in any sterile, biocompatible pharmaceutical carrier including, but not limited to, saline, buffered saline, dextrose, and water. The compositions may be administered to a patient alone, or in combination with other agents, drugs, or hormones.

The pharmaceutical compositions utilized in this invention may be administered by any number of routes including, but not limited to, oral, intravenous, intramuscular, intra-arterial, intramedullary, intrathecal, intraventricular, transdermal, subcutaneous, intraperitoneal, intranasal, enteral, topical, sublingual, or rectal means.

In addition to the active ingredients, these pharmaceutical compositions may contain suitable pharmaceutically-acceptable carriers comprising excipients and auxiliaries which facilitate processing of the active compounds into preparations which can be used pharmaceutically. Further details on techniques for formulation and administration may be found in the latest edition of Remington's Pharmaceutical Sciences (Maack Publishing Co., Easton, PA).

Pharmaceutical compositions for oral administration can be formulated using pharmaceutically acceptable carriers well known in the art in dosages suitable for oral administration. Such carriers enable the pharmaceutical compositions to be formulated as tablets, pills, dragees, capsules, liquids, gels, syrups, slurries, suspensions, and the like, for ingestion by the patient.

Pharmaceutical preparations for oral use can be obtained through combining active

compounds with solid excipient and processing the resultant mixture of granules (optionally, after grinding) to obtain tablets or dragee cores. Suitable auxiliaries can be added, if desired. Suitable excipients include carbohydrate or protein fillers, such as sugars, including lactose, sucrose, mannitol, and sorbitol; starch from corn, wheat, rice, potato, or other plants; cellulose, such as methyl cellulose, hydroxypropylmethyl-cellulose, or sodium carboxymethylcellulose; gums, including arabic and tragacanth; and proteins, such as gelatin and collagen. If desired, disintegrating or solubilizing agents may be added, such as the cross-linked polyvinyl pyrrolidone, agar, and alginic acid or a salt thereof, such as sodium alginate.

Dragee cores may be used in conjunction with suitable coatings, such as concentrated sugar solutions, which may also contain gum arabic, talc, polyvinylpyrrolidone, carbopol gel, polyethylene glycol, and/or titanium dioxide, lacquer solutions, and suitable organic solvents or solvent mixtures. Dyestuffs or pigments may be added to the tablets or dragee coatings for product identification or to characterize the quantity of active compound, i.e., dosage.

Pharmaceutical preparations which can be used orally include push-fit capsules made of gelatin, as well as soft, sealed capsules made of gelatin and a coating, such as glycerol or sorbitol. Push-fit capsules can contain active ingredients mixed with fillers or binders, such as lactose or starches, lubricants, such as talc or magnesium stearate, and, optionally, stabilizers. In soft capsules, the active compounds may be dissolved or suspended in suitable liquids, such as fatty oils, liquid, or liquid polyethylene glycol with or without stabilizers.

Pharmaceutical formulations suitable for parenteral administration may be formulated in aqueous solutions, preferably in physiologically compatible buffers such as Hanks' solution, Ringer's solution, or physiologically buffered saline. Aqueous injection suspensions may contain substances which increase the viscosity of the suspension, such as sodium carboxymethyl cellulose, sorbitol, or dextran. Additionally, suspensions of the active compounds may be prepared as appropriate oily injection suspensions. Suitable lipophilic solvents or vehicles include fatty oils, such as sesame oil, or synthetic fatty acid esters, such as ethyl oleate, triglycerides, or liposomes. Non-lipid polycationic amino polymers may also be used for delivery. Optionally, the suspension may also contain suitable stabilizers or agents to increase the solubility of the compounds and allow for the preparation of highly concentrated solutions.

For topical or nasal administration, penetrants appropriate to the particular barrier to be permeated are used in the formulation. Such penetrants are generally known in the art.

The pharmaceutical compositions of the present invention may be manufactured in a manner that is known in the art, e.g., by means of conventional mixing, dissolving, granulating,

dragee-making, levigating, emulsifying, encapsulating, entrapping, or lyophilizing processes.

The pharmaceutical composition may be provided as a salt and can be formed with many acids, including but not limited to, hydrochloric, sulfuric, acetic, lactic, tartaric, malic, and succinic acids. Salts tend to be more soluble in aqueous or other protonic solvents than are the corresponding free base forms. In other cases, the preferred preparation may be a lyophilized powder which may contain any or all of the following: 1 mM to 50 mM histidine, 0.1% to 2% sucrose, and 2% to 7% mannitol, at a pH range of 4.5 to 5.5, that is combined with buffer prior to use.

After pharmaceutical compositions have been prepared, they can be placed in an appropriate container and labeled for treatment of an indicated condition. For administration of GAPIP, such labeling would include amount, frequency, and method of administration.

Pharmaceutical compositions suitable for use in the invention include compositions wherein the active ingredients are contained in an effective amount to achieve the intended purpose. The determination of an effective dose is well within the capability of those skilled in the art.

For any compound, the therapeutically effective dose can be estimated initially either in cell culture assays, e.g., of neoplastic cells or in animal models such as mice, rats, rabbits, dogs, or pigs. An animal model may also be used to determine the appropriate concentration range and route of administration. Such information can then be used to determine useful doses and routes for administration in humans.

A therapeutically effective dose refers to that amount of active ingredient, for example GAPIP or fragments thereof, antibodies of GAPIP, and agonists, antagonists or inhibitors of GAPIP, which ameliorates the symptoms or condition. Therapeutic efficacy and toxicity may be determined by standard pharmaceutical procedures in cell cultures or with experimental animals, such as by calculating the ED<sub>50</sub> (the dose therapeutically effective in 50% of the population) or LD<sub>50</sub> (the dose lethal to 50% of the population) statistics. The dose ratio of toxic to therapeutic effects is the therapeutic index, and it can be expressed as the LD<sub>50</sub>/ED<sub>50</sub> ratio. Pharmaceutical compositions which exhibit large therapeutic indices are preferred. The data obtained from cell culture assays and animal studies are used to formulate a range of dosage for human use. The dosage contained in such compositions is preferably within a range of circulating concentrations that includes the ED<sub>50</sub> with little or no toxicity. The dosage varies within this range depending upon the dosage form employed, the sensitivity of the patient, and the route of administration.

The exact dosage will be determined by the practitioner, in light of factors related to the subject requiring treatment. Dosage and administration are adjusted to provide sufficient levels of the active moiety or to maintain the desired effect. Factors which may be taken into account include the

severity of the disease state, the general health of the subject, the age, weight, and gender of the subject, time and frequency of administration, drug combination(s), reaction sensitivities, and response to therapy. Long-acting pharmaceutical compositions may be administered every 3 to 4 days, every week, or biweekly depending on the half-life and clearance rate of the particular formulation.

5 Normal dosage amounts may vary from about 0.1  $\mu$ g to 100,000  $\mu$ g, up to a total dose of about 1 gram, depending upon the route of administration. Guidance as to particular dosages and methods of delivery is provided in the literature and generally available to practitioners in the art. Those skilled in the art will employ different formulations for nucleotides than for proteins or their inhibitors. Similarly, delivery of polynucleotides or polypeptides will be specific to particular cells, conditions,  
10 locations, etc.

## DIAGNOSTICS

In another embodiment, antibodies which specifically bind GAPIP may be used for the diagnosis of disorders characterized by expression of GAPIP, or in assays to monitor patients being treated with GAPIP or agonists, antagonists, or inhibitors of GAPIP. Antibodies useful for diagnostic purposes may be prepared in the same manner as described above for therapeutics. Diagnostic assays for GAPIP include methods which utilize the antibody and a label to detect GAPIP in human body fluids or in extracts of cells or tissues. The antibodies may be used with or without modification, and may be labeled by covalent or non-covalent attachment of a reporter molecule. A wide variety of reporter molecules, several of which are described above, are known in the art and may be used.

A variety of protocols for measuring GAPIP, including ELISAs, RIAs, and FACS, are known in the art and provide a basis for diagnosing altered or abnormal levels of GAPIP expression. Normal or standard values for GAPIP expression are established by combining body fluids or cell extracts taken from normal mammalian subjects, preferably human, with antibody to GAPIP under conditions suitable for complex formation. The amount of standard complex formation may be quantitated by various methods, preferably by photometric means. Quantities of GAPIP expressed in subject samples, control and disease, from biopsied tissues are compared with the standard values. Deviation between standard and subject values establishes the parameters for diagnosing disease.  
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In another embodiment of the invention, the polynucleotides encoding GAPIP may be used for diagnostic purposes. The polynucleotides which may be used include oligonucleotide sequences, complementary RNA and DNA molecules, and PNAs. The polynucleotides may be used to detect and quantitate gene expression in biopsied tissues in which expression of GAPIP may be correlated with disease. The diagnostic assay may be used to determine absence, presence, and excess expression of  
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GAPIP, and to monitor regulation of GAPIP levels during therapeutic intervention.

In one aspect, hybridization with PCR probes which are capable of detecting polynucleotide sequences, including genomic sequences, encoding GAPIP or closely related molecules may be used to identify nucleic acid sequences which encode GAPIP. The specificity of the probe, whether it is made from a highly specific region, e.g., the 5' regulatory region, or from a less specific region, e.g., a conserved motif, and the stringency of the hybridization or amplification (maximal, high, intermediate, or low), will determine whether the probe identifies only naturally occurring sequences encoding GAPIP, allelic variants, or related sequences.

Probes may also be used for the detection of related sequences, and should preferably have at least 50% sequence identity to any of the GAPIP encoding sequences. The hybridization probes of the subject invention may be DNA or RNA and may be derived from the sequence of SEQ ID NO:2 or from genomic sequences including promoters, enhancers, and introns of the GAPIP gene.

Means for producing specific hybridization probes for DNAs encoding GAPIP include the cloning of polynucleotide sequences encoding GAPIP or GAPIP derivatives into vectors for the production of mRNA probes. Such vectors are known in the art, are commercially available, and may be used to synthesize RNA probes *in vitro* by means of the addition of the appropriate RNA polymerases and the appropriate labeled nucleotides. Hybridization probes may be labeled by a variety of reporter groups, for example, by radionuclides such as  $^{32}\text{P}$  or  $^{35}\text{S}$ , or by enzymatic labels, such as alkaline phosphatase coupled to the probe via avidin/biotin coupling systems, and the like.

Polynucleotide sequences encoding GAPIP may be used for the diagnosis of a disorder associated with expression of GAPIP. Examples of such a disorder include, but are not limited to, a reproductive disorder, such as, disorders of prolactin production; infertility, including tubal disease, ovulatory defects, and endometriosis; disruptions of the estrous cycle, disruptions of the menstrual cycle, polycystic ovary syndrome, ovarian hyperstimulation syndrome, endometrial and ovarian tumors, uterine fibroids, autoimmune disorders, ectopic pregnancies, and teratogenesis; cancer of the breast, fibrocystic breast disease, and galactorrhea; disruptions of spermatogenesis, abnormal sperm physiology, cancer of the testis, cancer of the prostate, benign prostatic hyperplasia, prostatitis, Peyronie's disease, carcinoma of the male breast, and gynecomastia; a developmental disorder, such as, renal tubular acidosis, anemia, Cushing's syndrome, achondroplastic dwarfism, Duchenne and Becker muscular dystrophy, epilepsy, gonadal dysgenesis, WAGR syndrome (Wilms' tumor, aniridia, genitourinary abnormalities, and mental retardation), Smith-Magenis syndrome, myelodysplastic syndrome, hereditary mucoepithelial dysplasia, hereditary keratodermas, hereditary neuropathies such as Charcot-Marie-Tooth disease and neurofibromatosis, hypothyroidism, hydrocephalus, seizure

disorders such as Syndenham's chorea and cerebral palsy, spinal bifida, congenital glaucoma, cataract, and sensorineural hearing loss; a neoplastic disorder, such as, adenocarcinoma, leukemia, lymphoma, melanoma, myeloma, sarcoma, teratocarcinoma, and, in particular, cancers of the adrenal gland, bladder, bone, bone marrow, brain, breast, cervix, gall bladder, ganglia, gastrointestinal tract, heart, 5 kidney, liver, lung, muscle, ovary, pancreas, parathyroid, penis, prostate, salivary glands, skin, spleen, testis, thymus, thyroid, and uterus; and an immunological disorder, such as, acquired immunodeficiency syndrome (AIDS), Addison's disease, adult respiratory distress syndrome, allergies, ankylosing spondylitis, amyloidosis, anemia, asthma, atherosclerosis, autoimmune hemolytic anemia, autoimmune thyroiditis, bronchitis, cholecystitis, contact dermatitis, Crohn's 10 disease, atopic dermatitis, dermatomyositis, diabetes mellitus, emphysema, episodic lymphopenia with lymphocytotoxins, erythroblastosis fetalis, erythema nodosum, atrophic gastritis, glomerulonephritis, Goodpasture's syndrome, gout, Graves' disease, Hashimoto's thyroiditis, hypereosinophilia, irritable bowel syndrome, multiple sclerosis, myasthenia gravis, myocardial or pericardial inflammation, osteoarthritis, osteoporosis, pancreatitis, polymyositis, psoriasis, Reiter's syndrome, rheumatoid arthritis, scleroderma, Sjögren's syndrome, systemic anaphylaxis, systemic lupus erythematosus, systemic sclerosis, thrombocytopenic purpura, ulcerative colitis, uveitis, Werner syndrome, complications of cancer, hemodialysis, and extracorporeal circulation, viral, bacterial, fungal, parasitic, protozoal, and helminthic infections, and trauma; and arteriosclerosis, bursitis, cirrhosis, hepatitis, mixed connective tissue disease (MCTD), myelofibrosis, paroxysmal nocturnal hemoglobinuria, polycythemia vera, and primary thrombocythemia. The polynucleotide sequences encoding GAPIP may be used in Southern or Northern analysis, dot blot, or other membrane-based technologies; in PCR technologies; in dipstick, pin, and ELISA assays; and in microarrays utilizing fluids or tissues from patients to detect altered GAPIP expression. Such qualitative or quantitative methods are well known in the art.

25 In a particular aspect, the nucleotide sequences encoding GAPIP may be useful in assays that detect the presence of associated disorders, particularly those mentioned above. The nucleotide sequences encoding GAPIP may be labeled by standard methods and added to a fluid or tissue sample from a patient under conditions suitable for the formation of hybridization complexes. After a suitable incubation period, the sample is washed and the signal is quantitated and compared with a standard 30 value. If the amount of signal in the patient sample is significantly altered in comparison to a control sample then the presence of altered levels of nucleotide sequences encoding GAPIP in the sample indicates the presence of the associated disorder. Such assays may also be used to evaluate the efficacy of a particular therapeutic treatment regimen in animal studies, in clinical trials, or to monitor

the treatment of an individual patient.

In order to provide a basis for the diagnosis of a disorder associated with expression of GAPIP, a normal or standard profile for expression is established. This may be accomplished by combining body fluids or cell extracts taken from normal subjects, either animal or human, with a sequence, or a fragment thereof, encoding GAPIP, under conditions suitable for hybridization or amplification. Standard hybridization may be quantified by comparing the values obtained from normal subjects with values from an experiment in which a known amount of a substantially purified polynucleotide is used. Standard values obtained in this manner may be compared with values obtained from samples from patients who are symptomatic for a disorder. Deviation from standard values is used to establish the presence of a disorder.

Once the presence of a disorder is established and a treatment protocol is initiated, hybridization assays may be repeated on a regular basis to determine if the level of expression in the patient begins to approximate that which is observed in the normal subject. The results obtained from successive assays may be used to show the efficacy of treatment over a period ranging from several days to months.

With respect to cancer, the presence of a relatively high amount of transcript in biopsied tissue from an individual may indicate a predisposition for the development of the disease, or may provide a means for detecting the disease prior to the appearance of actual clinical symptoms. A more definitive diagnosis of this type may allow health professionals to employ preventative measures or aggressive treatment earlier thereby preventing the development or further progression of the cancer.

Additional diagnostic uses for oligonucleotides designed from the sequences encoding GAPIP may involve the use of PCR. These oligomers may be chemically synthesized, generated enzymatically, or produced in vitro. Oligomers will preferably contain a fragment of a polynucleotide encoding GAPIP, or a fragment of a polynucleotide complementary to the polynucleotide encoding GAPIP, and will be employed under optimized conditions for identification of a specific gene or condition. Oligomers may also be employed under less stringent conditions for detection or quantitation of closely related DNA or RNA sequences.

Methods which may also be used to quantitate the expression of GAPIP include radiolabeling or biotinylation nucleotides, coamplification of a control nucleic acid, and interpolating results from standard curves. (See, e.g., Melby, P.C. et al. (1993) J. Immunol. Methods 159:235-244; and Duplaa, C. et al. (1993) Anal. Biochem. 212:229-236.) The speed of quantitation of multiple samples may be accelerated by running the assay in an ELISA format where the oligomer of interest is presented in various dilutions and a spectrophotometric or colorimetric response gives rapid quantitation.

In further embodiments, oligonucleotides or longer fragments derived from any of the polynucleotide sequences described herein may be used as targets in a microarray. The microarray can be used to monitor the expression level of large numbers of genes simultaneously and to identify genetic variants, mutations, and polymorphisms. This information may be used to determine gene function, to understand the genetic basis of a disorder, to diagnose a disorder, and to develop and monitor the activities of therapeutic agents.

Microarrays may be prepared, used, and analyzed using methods known in the art. (See, e.g., Brennan, T.M. et al. (1995) U.S. Patent No. 5,474,796; Schena, M. et al. (1996) Proc. Natl. Acad. Sci. 93:10614-10619; Baldeschweiler et al. (1995) PCT application WO95/251116; Shalon, D. et al. (1995) PCT application WO95/35505; Heller, R.A. et al. (1997) Proc. Natl. Acad. Sci. 94:2150-2155; and Heller, M.J. et al. (1997) U.S. Patent No. 5,605,662.)

In another embodiment of the invention, nucleic acid sequences encoding GAPIP may be used to generate hybridization probes useful in mapping the naturally occurring genomic sequence. The sequences may be mapped to a particular chromosome, to a specific region of a chromosome, or to artificial chromosome constructions, e.g., human artificial chromosomes (HACs), yeast artificial chromosomes (YACs), bacterial artificial chromosomes (BACs), bacterial P1 constructions, or single chromosome cDNA libraries. (See, e.g., Price, C.M. (1993) Blood Rev. 7:127-134; and Trask, B.J. (1991) Trends Genet. 7:149-154.)

Fluorescent in situ hybridization (FISH) may be correlated with other physical chromosome mapping techniques and genetic map data. (See, e.g., Heinz-Ulrich, et al. (1995) in Meyers, R.A. (ed.) Molecular Biology and Biotechnology, VCH Publishers New York, NY, pp. 965-968.) Examples of genetic map data can be found in various scientific journals or at the Online Mendelian Inheritance in Man (OMIM) site. Correlation between the location of the gene encoding GAPIP on a physical chromosomal map and a specific disorder, or a predisposition to a specific disorder, may help define the region of DNA associated with that disorder. The nucleotide sequences of the invention may be used to detect differences in gene sequences among normal, carrier, and affected individuals.

In situ hybridization of chromosomal preparations and physical mapping techniques, such as linkage analysis using established chromosomal markers, may be used for extending genetic maps. Often the placement of a gene on the chromosome of another mammalian species, such as mouse, may reveal associated markers even if the number or arm of a particular human chromosome is not known. New sequences can be assigned to chromosomal arms by physical mapping. This provides valuable information to investigators searching for disease genes using positional cloning or other gene discovery techniques. Once the disease or syndrome has been crudely localized by genetic linkage to

a particular genomic region, e.g., ataxia-telangiectasia to 11q22-23, any sequences mapping to that area may represent associated or regulatory genes for further investigation. (See, e.g., Gatti, R.A. et al. (1988) *Nature* 336:577-580.) The nucleotide sequence of the subject invention may also be used to detect differences in the chromosomal location due to translocation, inversion, etc., among normal, carrier, or affected individuals.

In another embodiment of the invention, GAPIP, its catalytic or immunogenic fragments, or oligopeptides thereof can be used for screening libraries of compounds in any of a variety of drug screening techniques. The fragment employed in such screening may be free in solution, affixed to a solid support, borne on a cell surface, or located intracellularly. The formation of binding complexes between GAPIP and the agent being tested may be measured.

Another technique for drug screening provides for high throughput screening of compounds having suitable binding affinity to the protein of interest. (See, e.g., Geysen, et al. (1984) PCT application WO84/03564.) In this method, large numbers of different small test compounds are synthesized on a solid substrate, such as plastic pins or some other surface. The test compounds are reacted with GAPIP, or fragments thereof, and washed. Bound GAPIP is then detected by methods well known in the art. Purified GAPIP can also be coated directly onto plates for use in the aforementioned drug screening techniques. Alternatively, non-neutralizing antibodies can be used to capture the peptide and immobilize it on a solid support.

In another embodiment, one may use competitive drug screening assays in which neutralizing antibodies capable of binding GAPIP specifically compete with a test compound for binding GAPIP. In this manner, antibodies can be used to detect the presence of any peptide which shares one or more antigenic determinants with GAPIP.

In additional embodiments, the nucleotide sequences which encode GAPIP may be used in any molecular biology techniques that have yet to be developed, provided the new techniques rely on properties of nucleotide sequences that are currently known, including, but not limited to, such properties as the triplet genetic code and specific base pair interactions.

The examples below are provided to illustrate the subject invention and are not included for the purpose of limiting the invention.

## EXAMPLES

### I. cDNA Library Construction

The UTRSNOT02 cDNA library was constructed from uterus tissue obtained from a 34-year old Caucasian female (specimen #0047A) during a vaginal hysterectomy. Pathology indicated no

diagnostic abnormality in the uterus or cervix. However, the left ovarian tissue showed dilated follicular cystis, all embedded. Patient history included the diagnoses of dysmenorrhea, dyspareunia, hemorrhoids and alcohol use. The patient was not taking any medications. Family history included malignant stomach neoplasm in the mother; benign large bowel neoplasm in the father; congenital heart anomaly, irritable bowel syndrome, and ulcerative colitis in the siblings; colon cancer in the paternal aunt at age sixty and colon cancer in paternal uncle at age eleven; and cerebrovascular disease, type II diabetes, and depression in the grandparents.

The frozen tissue was homogenized and lysed using a Brinkmann Homogenizer Polytron PT-3000 (Brinkmann Instruments, Westbury, NY) in guanidinium isothiocyanate solution. The lysate was centrifuged over a 5.7 M CsCl cushion using an SW28 rotor in an L8-70M ultracentrifuge (Beckman Coulter, Fullerton CA) for 18 hours at 25,000 rpm at ambient temperature. The RNA was extracted with acid phenol pH 4.7, precipitated using 0.3 M sodium acetate and 2.5 volumes of ethanol, resuspended in RNase-free water, and DNase treated at 37 °C. RNA extraction and precipitation were repeated as before. The mRNA was then isolated using the QIAGEN OLIGOTEX kit (QIAGEN Inc., Chatsworth CA) and used to construct the cDNA library.

The mRNA was handled according to the recommended protocols in the SUPERSCRIPT plasmid system (Life Technologies). cDNA synthesis was initiated with a NotI-oligo d(T) primer. Double-stranded cDNA was blunted, ligated to Sall adaptors, digested with NotI, fractionated on a SEPHAROSE CL4B column (Catalog #275105-01, APB), and those cDNAs exceeding 400 bp were ligated into the NotI and Sall sites of the PSPORT 1 vector. The plasmid PSPORT 1 was subsequently transformed into DH5 $\alpha$  competent cells (Catalog #18258-012, Life Technologies).

## II Isolation and Sequencing of cDNA Clones

Plasmid DNA was released from the cells and purified using the REAL PREP 96 plasmid kit (QIAGEN Inc.). The recommended protocol was employed except for the following changes: 1) the bacteria were cultured in 1 ml of sterile TERRIFIC BROTH (Life Technologies) with carbenicillin at 25 mg/l and glycerol at 0.4%; 2) after inoculation, the cultures were incubated for 19 hours and at the end of incubation, the cells were lysed with 0.3 ml of lysis buffer; and 3) following isopropanol precipitation, the plasmid DNA pellet was resuspended in 0.1 ml of distilled water. After the last step in the protocol, samples were transferred to a 96-well block for storage at 4 ° C.

The cDNAs were sequenced by the method of Sanger et al. (1975, J. Mol. Biol. 94:441f), using a Hamilton Micro Lab 2200 (Hamilton, Reno, NV) in combination with the DNA ENGINE thermal cyclers (MJ Research) and Applied Biosystems 377 DNA Sequencing Systems; and the

reading frame was determined.

### III. Similarity Searching of cDNA Clones and Their Dduced Proteins

The nucleotide sequences and/or amino acid sequences of the Sequence Listing were used to query sequences in the GenBank, SwissProt, BLOCKS, and Pima II databases. These databases, which contain previously identified and annotated sequences, were searched for regions of similarity using BLAST (Basic Local Alignment Search Tool). (See, e.g., Altschul, S.F. (1993) J. Mol. Evol 36:290-300; and Altschul et al. (1990) J. Mol. Biol. 215:403-410.)

BLAST produced alignments of both nucleotide and amino acid sequences to determine sequence similarity. Because of the local nature of the alignments, BLAST was especially useful in determining exact matches or in identifying homologs which may be of prokaryotic (bacterial) or eukaryotic (animal, fungal, or plant) origin. Other algorithms could have been used when dealing with primary sequence patterns and secondary structure gap penalties. (See, e.g., Smith, T. et al. (1992) Protein Engineering 5:35-51.) The sequences disclosed in this application have lengths of at least 49 nucleotides and have no more than 12% uncalled bases (where N is recorded rather than A, C, G, or T).

The BLAST approach searched for matches between a query sequence and a database sequence. BLAST evaluated the statistical significance of any matches found, and reported only those matches that satisfy the user-selected threshold of significance. In this application, threshold was set at  $10^{-25}$  for nucleotides and  $10^{-8}$  for peptides.

Incyte nucleotide sequences were searched against the GenBank databases for primate (pri), rodent (rod), and other mammalian sequences (mam), and deduced amino acid sequences from the same clones were then searched against GenBank functional protein databases, mammalian (mamp), vertebrate (vrtp), and eukaryote (eukp), for similarity.

Additionally, sequences identified from cDNA libraries may be analyzed to identify those gene sequences encoding conserved protein motifs using an appropriate analysis program, e.g., BLOCKS. BLOCKS is a weighted matrix analysis algorithm based on short amino acid segments, or blocks, compiled from the PROSITE database. (Bairoch, A. et al. (1997) Nucleic Acids Res. 25:217-221.) The BLOCKS algorithm is useful for classifying genes with unknown functions. (Henikoff S. And Henikoff G.J., Nucleic Acids Research (1991) 19:6565-6572.) Blocks, which are 3-60 amino acids in length, correspond to the most highly conserved regions of proteins. The BLOCKS algorithm compares a query sequence with a weighted scoring matrix of blocks in the BLOCKS database. Blocks in the BLOCKS database are calibrated against protein sequences with known functions from

the SWISS-PROT database to determine the stochastic distribution of matches. Similar databases such as PRINTS, a protein fingerprint database, are also searchable using the BLOCKS algorithm. (Attwood, T. K. et al. (1997) J. Chem. Inf. Comput. Sci. 37:417-424.) PRINTS is based on non-redundant sequences obtained from sources such as SWISS-PROT, GenBank, PIR, and NRL-3D.

5        The BLOCKS algorithm searches for matches between a query sequence and the BLOCKS or PRINTS database and evaluates the statistical significance of any matches found. Matches from a BLOCKS or PRINTS search can be evaluated on two levels, local similarity and global similarity. The degree of local similarity is measured by scores, and the extent of global similarity is measured by score ranking and probability values. A score of 1000 or greater for a BLOCKS match of highest 10 ranking indicates that the match falls within the 0.5 percentile level of false positives when the matched block is calibrated against SWISS-PROT. Likewise, a probability value of less than  $1.0 \times 10^{-3}$  indicates that the match would occur by chance no more than one time in every 1000 searches. Only those matches with a cutoff score of 1000 or greater and a cutoff probability value of  $1.0 \times 10^{-3}$  or less are considered in the functional analyses of the protein sequences in the Sequence Listing.

15        Nucleic and amino acid sequences of the Sequence Listing may also be analyzed using PFAM. PFAM is a Hidden Markov Model (HMM) based protocol useful in protein family searching. HMM is a probabilistic approach which analyzes consensus primary structures of gene families. (See, e.g., Eddy, S.R. (1996) Cur. Opin. Str. Biol. 6:361-365.)

20        The PFAM database contains protein sequences of 527 protein families gathered from publicly available sources, e.g., SWISS-PROT and PROSITE. PFAM searches for well characterized protein domain families using two high-quality alignment routines, seed alignment and full alignment. (See, e.g., Sonnhammer, E.L.L. et al. (1997) Proteins 28:405-420.) The seed alignment utilizes the hmmls program, a program that searches for local matches, and a non-redundant set of the PFAM database. The full alignment utilizes the hmmfs program, a program that searches for multiple 25 fragments in long sequences, e.g., repeats and motifs, and all sequences in the PFAM database. A result or score of 100 "bits" can signify that it is  $2^{100}$ -fold more likely that the sequence is a true match to the model or comparison sequence. Cutoff scores which range from 10 to 50 bits are generally used for individual protein families using the SWISS-PROT sequences as model or comparison sequences.

30        Two other algorithms, SIGPEPT and TM, both based on the HMM algorithm described above (see, e.g., Eddy, supra; and Sonnhammer, supra), identify potential signal sequences and transmembrane domains, respectively. SIGPEPT was created using protein sequences having signal sequence annotations derived from SWISS-PROT. It contains about 1413 non-redundant signal sequences ranging in length from 14 to 36 amino acid residues. TM was created similarly using

transmembrane domain annotations. It contains about 453 non-redundant transmembrane sequences encompassing 1579 transmembrane domain segments. Suitable HMM models were constructed using the above sequences and were refined with known SWISS-PROT signal peptide sequences or transmembrane domain sequences until a high correlation coefficient, a measurement of the correctness of the analysis, was obtained. Using the protein sequences from the SWISS-PROT database as a test set, a cutoff score of 11 bits, as determined above, correlated with 91-94% true-positives and about 4.1% false-positives, yielding a correlation coefficient of about 0.87-0.90 for SIGPEPT. A score of 11 bits for TM will typically give the following results: 75% true positives; 1.72% false positives; and a correlation coefficient of 0.76. Each search evaluates the statistical significance of any matches found and reports only those matches that score at least 11 bits.

#### IV. Northern Analysis

Northern analysis is a laboratory technique used to detect the presence of a transcript of a gene and involves the hybridization of a labeled nucleotide sequence to a membrane on which RNAs from a particular cell type or tissue have been bound. (See, e.g., Sambrook, *supra*, ch. 7; and Ausubel, *supra*, ch. 4 and 16.)

Analogous computer techniques applying BLAST are used to search for identical or related molecules in nucleotide databases such as GenBank or LIFESEQ database (Incyte Genomics, Palo Alto CA). This analysis is much faster than multiple membrane-based hybridizations. In addition, the sensitivity of the computer search can be modified to determine whether any particular match is categorized as exact or similar.

The basis of the search is the product score, which is defined as:

$$\frac{\% \text{ sequence identity} \times \% \text{ maximum BLAST score}}{100}$$

The product score takes into account both the degree of similarity between two sequences and the length of the sequence match. For example, with a product score of 40, the match will be exact within a 1% to 2% error, and, with a product score of 70, the match will be exact. Similar molecules are usually identified by selecting those which show product scores between 15 and 40, although lower scores may identify related molecules.

The results of Northern analysis are reported as a list of libraries in which the transcript encoding GAPIP occurs. Abundance and percent abundance are also reported. Abundance directly reflects the number of times a particular transcript is represented in a cDNA library, and percent abundance is abundance divided by the total number of sequences examined in the cDNA library.

**V. Extension of GAPIP Encoding Polynucleotides**

The nucleic acid sequence of Incyte Clone 688183 was used to design oligonucleotide primers for extending a partial nucleotide sequence to full length. One primer was synthesized to initiate extension of an antisense polynucleotide, and the other was synthesized to initiate extension of a sense polynucleotide. Primers were used to facilitate the extension of the known sequence "outward" generating amplicons containing new unknown nucleotide sequence for the region of interest. The initial primers were designed from the cDNA using OLIGO 4.06 (National Biosciences), or another appropriate program, to be about 22 to 30 nucleotides in length, to have a GC content of about 50% or more, and to anneal to the target sequence at temperatures of about 68°C to about 72°C. Any stretch of nucleotides which would result in hairpin structures and primer-primer dimerizations was avoided.

Selected human cDNA libraries (Life Technologies) were used to extend the sequence. If more than one extension is necessary or desired, additional sets of primers are designed to further extend the known region.

High fidelity amplification was obtained by following the instructions for the XL-PCR kit (Applied Biosystems) and thoroughly mixing the enzyme and reaction mix. PCR was performed using the DNA ENGINE thermal cyclers (MJ Research), beginning with 40 pmol of each primer and the recommended concentrations of all other components of the kit, with the following parameters:

Step 1	94° C for 1 min (initial denaturation)
Step 2	65° C for 1 min
Step 3	68° C for 6 min
Step 4	94° C for 15 sec
Step 5	65° C for 1 min
Step 6	68° C for 7 min
Step 7	Repeat steps 4 through 6 for an additional 15 cycles
Step 8	94° C for 15 sec
Step 9	65° C for 1 min
Step 10	68° C for 7:15 min
Step 11	Repeat steps 8 through 10 for an additional 12 cycles
Step 12	72° C for 8 min
Step 13	4° C (and holding)

A 5  $\mu$ l to 10  $\mu$ l aliquot of the reaction mixture was analyzed by electrophoresis on a low concentration (about 0.6% to 0.8%) agarose mini-gel to determine which reactions were successful in extending the sequence. Bands thought to contain the largest products were excised from the gel, purified using QIAQUICK (QIAGEN Inc.), and trimmed of overhangs using Klenow enzyme to facilitate religation and cloning.

After ethanol precipitation, the products were redissolved in 13  $\mu$ l of ligation buffer, 1  $\mu$ l T4-DNA ligase (15 units) and 1  $\mu$ l T4 polynucleotide kinase were added, and the mixture was incubated at

room temperature for 2 to 3 hours, or overnight at 16° C. Competent *E. coli* cells (in 40  $\mu$ l of appropriate media) were transformed with 3  $\mu$ l of ligation mixture and cultured in 80  $\mu$ l of SOC medium. (See, e.g., Sambrook, *supra*, Appendix A, p. 2.) After incubation for one hour at 37°C, the *E. coli* mixture was plated on Luria Bertani (LB) agar (See, e.g., Sambrook, *supra*, Appendix A, p. 1) containing carbenicillin (2x carb). The following day, several colonies were randomly picked from each plate and cultured in 150  $\mu$ l of liquid LB/2x carb medium placed in an individual well of an appropriate commercially-available sterile 96-well microtiter plate. The following day, 5  $\mu$ l of each overnight culture was transferred into a non-sterile 96-well plate and, after dilution 1:10 with water, 5  $\mu$ l from each sample was transferred into a PCR array.

For PCR amplification, 18  $\mu$ l of concentrated PCR reaction mix (3.3x) containing 4 units of rTth DNA polymerase, a vector primer, and one or both of the gene specific primers used for the extension reaction were added to each well. Amplification was performed using the following conditions:

Step 1	94° C for 60 sec
Step 2	94° C for 20 sec
Step 3	55° C for 30 sec
Step 4	72° C for 90 sec
Step 5	Repeat steps 2 through 4 for an additional 29 cycles
Step 6	72° C for 180 sec
Step 7	4° C (and holding)

Aliquots of the PCR reactions were run on agarose gels together with molecular weight markers. The sizes of the PCR products were compared to the original partial cDNAs, and appropriate clones were selected, ligated into plasmid, and sequenced.

In like manner, the nucleotide sequence of SEQ ID NO:2 is used to obtain 5' regulatory sequences using the procedure above, oligonucleotides designed for 5' extension, and an appropriate genomic library.

## VI. Labeling and Use of Individual Hybridization Probes

Hybridization probes derived from SEQ ID NO:2 are employed to screen cDNAs, genomic DNAs, or mRNAs. Although the labeling of oligonucleotides, consisting of about 20 base pairs, is specifically described, essentially the same procedure is used with larger nucleotide fragments. Oligonucleotides are designed using state-of-the-art software such as OLIGO 4.06 software (National Biosciences) and labeled by combining 50 pmol of each oligomer, 250  $\mu$ Ci of [ $\gamma$ -<sup>32</sup>P] adenosine triphosphate (Amersham, Chicago, IL), and T4 polynucleotide kinase (DuPont NEN, Boston, MA). The labeled oligonucleotides are substantially purified using a SEPHADEX G-25 superfine size

exclusion dextran bead column (APB). An aliquot containing  $10^7$  counts per minute of the labeled probe is used in a typical membrane-based hybridization analysis of human genomic DNA digested with one of the following endonucleases: AseI, BglII, EcoRI, PstI, Xba1, or PvuII (DuPont NEN, Boston, MA).

5       The DNA from each digest is fractionated on a 0.7% agarose gel and transferred to nylon membranes (Nytran Plus, Schleicher & Schuell, Durham, NH). Hybridization is carried out for 16 hours at 40°C. To remove nonspecific signals, blots are sequentially washed at room temperature under increasingly stringent conditions up to 0.1 x saline sodium citrate and 0.5% sodium dodecyl sulfate. After XOMAT AR film (Kodak, Rochester, NY) is exposed to the blots to film for several  
10 hours, hybridization patterns are compared visually.

## **VII. Microarrays**

A chemical coupling procedure and an ink jet device can be used to synthesize array elements on the surface of a substrate. (See, e.g., Baldeschweiler, *supra*.) An array analogous to a dot or slot blot may also be used to arrange and link elements to the surface of a substrate using thermal, UV, chemical, or mechanical bonding procedures. A typical array may be produced by hand or using available methods and machines and contain any appropriate number of elements. After hybridization, nonhybridized probes are removed and a scanner used to determine the levels and patterns of fluorescence. The degree of complementarity and the relative abundance of each probe which hybridizes to an element on the microarray may be assessed through analysis of the scanned images.  
20

Full-length cDNAs, Expressed Sequence Tags (ESTs), or fragments thereof may comprise the elements of the microarray. Fragments suitable for hybridization can be selected using software well known in the art such as LASERGENE. Full-length cDNAs, ESTs, or fragments thereof corresponding to one of the nucleotide sequences of the present invention, or selected at random from  
25 a cDNA library relevant to the present invention, are arranged on an appropriate substrate, e.g., a glass slide. The cDNA is fixed to the slide using, e.g., UV cross-linking followed by thermal and chemical treatments and subsequent drying. (See, e.g., Schena, M. et al. (1995) Science 270:467-470; and Shalon, D. et al. (1996) Genome Res. 6:639-645.) Fluorescent probes are prepared and used for hybridization to the elements on the substrate. The substrate is analyzed by procedures described  
30 above.

## **VIII. Complementary Polynucleotides**

Sequences complementary to the GAPIP-encoding sequences, or any parts thereof, are used

to detect, decrease, or inhibit expression of naturally occurring GAPIP. Although use of oligonucleotides comprising from about 15 to 30 base pairs is described, essentially the same procedure is used with smaller or with larger sequence fragments. Appropriate oligonucleotides are designed using OLIGO 4.06 software and the coding sequence of GAPIP. To inhibit transcription, a complementary oligonucleotide is designed from the most unique 5' sequence and used to prevent promoter binding to the coding sequence. To inhibit translation, a complementary oligonucleotide is designed to prevent ribosomal binding to the GAPIP-encoding transcript.

10      **IX. Expression of GAPIP**

Expression and purification of GAPIP is achieved using bacterial or virus-based expression systems. For expression of GAPIP in bacteria, cDNA is subcloned into an appropriate vector containing an antibiotic resistance gene and an inducible promoter that directs high levels of cDNA transcription. Examples of such promoters include, but are not limited to, the *trp-lac (tac)* hybrid promoter and the T5 or T7 bacteriophage promoter in conjunction with the *lac* operator regulatory element. Recombinant vectors are transformed into suitable bacterial hosts, e.g., BL21(DE3). Antibiotic resistant bacteria express GAPIP upon induction with isopropyl beta-D-thiogalactopyranoside (IPTG). Expression of GAPIP in eukaryotic cells is achieved by infecting insect or mammalian cell lines with recombinant Autographica californica nuclear polyhedrosis virus (AcMNPV), commonly known as baculovirus. The nonessential polyhedrin gene of baculovirus is replaced with cDNA encoding GAPIP by either homologous recombination or bacterial-mediated transposition involving transfer plasmid intermediates. Viral infectivity is maintained and the strong polyhedrin promoter drives high levels of cDNA transcription. Recombinant baculovirus is used to infect Spodoptera frugiperda (Sf9) insect cells in most cases, or human hepatocytes, in some cases. Infection of the latter requires additional genetic modifications to baculovirus. (See Engelhard, E. K. et al. (1994) Proc. Natl. Acad. Sci. USA 91:3224-3227; Sandig, V. et al. (1996) Hum. Gene Ther. 7:1937-1945.)

In most expression systems, GAPIP is synthesized as a fusion protein with, e.g., glutathione S-transferase (GST) or a peptide epitope tag, such as FLAG or 6-His, permitting rapid, single-step, affinity-based purification of recombinant fusion protein from crude cell lysates. GST, a 26-kilodalton enzyme from Schistosoma japonicum, enables the purification of fusion proteins on immobilized glutathione under conditions that maintain protein activity and antigenicity (Pharmacia, Piscataway, NJ). Following purification, the GST moiety can be proteolytically cleaved from GAPIP at

specifically engineered sites. FLAG, an 8-amino acid peptide, enables immunoaffinity purification using commercially available monoclonal and polyclonal anti-FLAG antibodies (Eastman Kodak, Rochester, NY). 6-His, a stretch of six consecutive histidine residues, enables purification on metal-chelate resins (QIAGEN Inc.). Methods for protein expression and purification are discussed in  
5 Ausubel, F. M. et al. (1995 and periodic supplements) Current Protocols in Molecular Biology, John Wiley & Sons, New York, NY, ch 10, 16. Purified GAPIP obtained by these methods can be used directly in the following activity assay.

#### X. Demonstration of GAPIP Activity

10 Protease inhibitory activity of GAPIP is measured by the inhibition of hydrolysis by trypsin of appropriate synthetic peptide substrates conjugated with various chromogenic molecules in which the degree of hydrolysis is quantitated by spectrophotometric (or fluorometric) absorption of the released chromophore (Beynon and Bond supra, pp.25-55). Peptide substrates are selected for optimal activity using prepared trypsin. Chromogens commonly used are 2-naphthylamine, 4-nitroaniline, and furylacrylic acid. Assays are performed at ambient temperature and contain an aliquot of trypsin, the appropriate substrate in a suitable buffer, and serial dilutions of purified GAPIP. Reactions are carried out in an optical cuvette and followed by the increase/decrease in absorbance or fluorescence of the chromogen released during hydrolysis of the peptide substrate. The baseline absorbance in the absence of GAPIP is proportional to the trypsin activity in the assay. Reduction in absorbance is proportional to the GAPIP activity in the assay.  
15  
20  
25  
30

#### XI. Functional Assays

GAPIP function is assessed by expressing the sequences encoding GAPIP at physiologically elevated levels in mammalian cell culture systems. cDNA is subcloned into a mammalian expression vector containing a strong promoter that drives high levels of cDNA expression. Vectors of choice include pCMV SPORT (Life Technologies) and pCR 3.1 (Invitrogen, Carlsbad, CA, both of which contain the cytomegalovirus promoter. 5-10  $\mu$ g of recombinant vector are transiently transfected into a human cell line, preferably of endothelial or hematopoietic origin, using either liposome formulations or electroporation. 1-2  $\mu$ g of an additional plasmid containing sequences encoding a marker protein are co-transfected. Expression of a marker protein provides a means to distinguish transfected cells from nontransfected cells and is a reliable predictor of cDNA expression from the recombinant vector. Marker proteins of choice include, e.g., Green Fluorescent Protein (GFP) (Clontech, Palo Alto, CA), CD64, or a CD64-GFP fusion protein. Flow cytometry (FCM), an  
30

automated, laser optics-based technique, is used to identify transfected cells expressing GFP or CD64-GFP, and to evaluate properties, for example, their apoptotic state. FCM detects and quantifies the uptake of fluorescent molecules that diagnose events preceding or coincident with cell death. These events include changes in nuclear DNA content as measured by staining of DNA with propidium iodide; changes in cell size and granularity as measured by forward light scatter and 90 degree side light scatter; down-regulation of DNA synthesis as measured by decrease in bromodeoxyuridine uptake; alterations in expression of cell surface and intracellular proteins as measured by reactivity with specific antibodies; and alterations in plasma membrane composition as measured by the binding of fluorescein-conjugated Annexin V protein to the cell surface. Methods in flow cytometry are discussed in Ormerod, M. G. (1994) *Flow Cytometry*, Oxford, New York, NY.

The influence of GAPIP on gene expression can be assessed using highly purified populations of cells transfected with sequences encoding GAPIP and either CD64 or CD64-GFP. CD64 and CD64-GFP are expressed on the surface of transfected cells and bind to conserved regions of human immunoglobulin G (IgG). Transfected cells are efficiently separated from nontransfected cells using magnetic beads coated with either human IgG or antibody against CD64 (DYNAL, Lake Success, NY). mRNA can be purified from the cells using methods well known by those of skill in the art. Expression of mRNA encoding GAPIP and other genes of interest can be analyzed by Northern analysis or microarray techniques.

## **XII. Production of GAPIP Specific Antibodies**

GAPIP substantially purified using polyacrylamide gel electrophoresis (PAGE)(see, e.g., Harrington, M.G. (1990) *Methods Enzymol.* 182:488-495), or other purification techniques, is used to immunize rabbits and to produce antibodies using standard protocols.

Alternatively, the GAPIP amino acid sequence is analyzed using LASERGENE software to determine regions of high immunogenicity, and a corresponding oligopeptide is synthesized and used to raise antibodies by means known to those of skill in the art. Methods for selection of appropriate epitopes, such as those near the C-terminus or in hydrophilic regions are well described in the art. (See, e.g., Ausubel *supra*, ch. 11.)

Typically, oligopeptides 15 residues in length are synthesized using an Applied Biosystems peptide synthesizer Model 431A using fmoc-chemistry and coupled to KLH (Sigma, St. Louis, MO) by reaction with N-maleimidobenzoyl-N-hydroxysuccinimide ester (MBS) to increase immunogenicity. (See, e.g., Ausubel *supra*.) Rabbits are immunized with the oligopeptide-KLH complex in complete Freund's adjuvant. Resulting antisera are tested for antipeptide activity by, for

example, binding the peptide to plastic, blocking with 1% BSA, reacting with rabbit antisera, washing, and reacting with radio-iodinated goat anti-rabbit IgG.

**XIII. Purification of Naturally Occurring GAPIP Using Specific Antibodies**

5       Naturally occurring or recombinant GAPIP is substantially purified by immunoaffinity chromatography using antibodies specific for GAPIP. An immunoaffinity column is constructed by covalently coupling anti-GAPIP antibody to an activated chromatographic resin, such as CNBr-activated SEPHAROSE (APB). After the coupling, the resin is blocked and washed according to the manufacturer's instructions.

10       Media containing GAPIP are passed over the immunoaffinity column, and the column is washed under conditions that allow the preferential absorbance of GAPIP (e.g., high ionic strength buffers in the presence of detergent). The column is eluted under conditions that disrupt antibody/GAPIP binding (e.g., a buffer of pH 2 to pH 3, or a high concentration of a chaotrope, such as urea or thiocyanate ion), and GAPIP is collected.

**XIV. Identification of Molecules Which Interact with GAPIP**

15       GAPIP, or biologically active fragments thereof, are labeled with  $^{125}\text{I}$  Bolton-Hunter reagent. (See, e.g., Bolton et al. (1973) Biochem. J. 133:529.) Candidate molecules previously arrayed in the wells of a multi-well plate are incubated with the labeled GAPIP, washed, and any wells with labeled GAPIP complex are assayed. Data obtained using different concentrations of GAPIP are used to calculate values for the number, affinity, and association of GAPIP with the candidate molecules.

20       Various modifications and variations of the described methods and systems of the invention will be apparent to those skilled in the art without departing from the scope and spirit of the invention. Although the invention has been described in connection with specific preferred embodiments, it should be understood that the invention as claimed should not be unduly limited to such specific embodiments. Indeed, various modifications of the described modes for carrying out the invention which are obvious to those skilled in molecular biology or related fields are intended to be within the scope of the following claims.

SEQUENCE LISTING

(1) GENERAL INFORMATION

(i) APPLICANT: Hillman, Jennifer L.  
Guegler, Karl J.  
Patterson, Chandra

(ii) TITLE OF THE INVENTION: GROWTH-ASSOCIATED TRYPSIN-TYPE  
INHIBITOR HEAVY CHAIN PRECURSOR

(iii) NUMBER OF SEQUENCES: 5

(iv) CORRESPONDENCE ADDRESS:

(A) ADDRESSEE: Incyte Pharmaceuticals, Inc.  
(B) STREET: 3174 Porter Drive  
(C) CITY: Palo Alto  
(D) STATE: CA  
(E) COUNTRY: USA  
(F) ZIP: 94304

(v) COMPUTER READABLE FORM:

(A) MEDIUM TYPE: Diskette  
(B) COMPUTER: IBM Compatible  
(C) OPERATING SYSTEM: DOS  
(D) SOFTWARE: Word Perfect 6.1/MS-DOS 6.2

(vi) CURRENT APPLICATION DATA:

(A) APPLICATION NUMBER: TO BE ASSIGNED  
(B) FILING DATE: HEREWITH  
(C) CLASSIFICATION:

(vii) PRIOR APPLICATION DATA:

(A) APPLICATION NUMBER:  
(B) FILING DATE:

(viii) ATTORNEY/AGENT INFORMATION:

(A) NAME: Cerrone, Michael C  
(B) REGISTRATION NUMBER: 39,132  
(C) REFERENCE/DOCKET NUMBER: PF-0505 US

(ix) TELECOMMUNICATION INFORMATION:

(A) TELEPHONE: 650-855-0555  
(B) TELEFAX: 650-845-4166  
(C) TELEX:

(2) INFORMATION FOR SEQ ID NO: 1:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 942 amino acids  
(B) TYPE: amino acid  
(C) STRANDEDNESS: single  
(D) TOPOLOGY: linear

(vii) IMMEDIATE SOURCE:

(A) LIBRARY: UTRSNOT02  
(B) CLONE: 688183

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 1 :

Met Leu Leu Leu Leu Gly Leu Cys Leu Gly Leu Ser Leu Cys Val  
                       5                    10                    15  
 Gly Ser Gln Glu Glu Ala Gln Ser Trp Gly His Ser Ser Glu Gln  
                       20                    25                    30  
 Asp Gly Leu Arg Val Pro Arg Gln Val Arg Leu Leu Gln Arg Leu  
                       35                    40                    45  
 Lys Thr Lys Pro Leu Met Thr Glu Phe Ser Val Lys Ser Thr Ile  
                       50                    55                    60  
 Ile Ser Arg Tyr Ala Phe Thr Thr Val Ser Cys Arg Met Leu Asn  
                       65                    70                    75  
 Arg Ala Ser Glu Asp Gln Asp Ile Glu Phe Gln Met Gln Ile Pro  
                       80                    85                    90  
 Ala Ala Ala Phe Ile Thr Asn Phe Thr Met Leu Ile Gly Asp Lys  
                       95                    100                   105  
 Val Tyr Gln Gly Glu Ile Thr Glu Arg Glu Lys Lys Ser Gly Asp  
                       110                    115                   120  
 Arg Val Lys Glu Lys Arg Asn Lys Thr Thr Glu Glu Asn Gly Glu  
                       125                    130                   135  
 Lys Gly Thr Glu Ile Phe Arg Ala Ser Ala Val Ile Pro Ser Lys  
                       140                    145                   150  
 Asp Lys Ala Ala Phe Phe Leu Ser Tyr Glu Glu Leu Leu Gln Arg  
                       155                    160                   165  
 Arg Leu Gly Lys Tyr Glu His Ser Ile Ser Val Arg Pro Gln Gln  
                       170                    175                   180  
 Leu Ser Gly Arg Leu Ser Val Asp Val Asn Ile Leu Glu Ser Ala  
                       185                    190                   195  
 Gly Ile Ala Ser Leu Glu Val Leu Pro Leu His Asn Ser Arg Gln  
                       200                    205                   210  
 Arg Gly Ser Gly Arg Gly Glu Asp Asp Ser Gly Pro Pro Pro Ser  
                       215                    220                   225  
 Thr Val Ile Asn Gln Asn Glu Thr Phe Ala Asn Ile Ile Phe Lys  
                       230                    235                   240  
 Pro Thr Val Val Gln Gln Ala Arg Ile Ala Gln Asn Gly Ile Leu  
                       245                    250                   255  
 Gly Asp Phe Ile Ile Arg Tyr Asp Val Asn Arg Glu Gln Ser Ile  
                       260                    265                   270  
 Gly Asp Ile Gln Val Leu Asn Gly Tyr Phe Val His Tyr Phe Ala  
                       275                    280                   285  
 Pro Lys Asp Leu Pro Pro Leu Pro Lys Asn Val Val Phe Val Leu  
                       290                    295                   300  
 Asp Ser Ser Ala Ser Met Val Gly Thr Lys Leu Arg Gln Thr Lys  
                       305                    310                   315  
 Asp Ala Leu Phe Thr Ile Leu His Asp Leu Arg Pro Gln Asp Arg  
                       320                    325                   330  
 Phe Ser Ile Ile Gly Phe Ser Asn Arg Ile Lys Val Trp Lys Asp  
                       335                    340                   345  
 His Leu Ile Ser Val Thr Pro Asp Ser Ile Arg Asp Gly Lys Val  
                       350                    355                   360  
 Tyr Ile His His Met Ser Pro Thr Gly Gly Thr Asp Ile Asn Gly  
                       365                    370                   375  
 Ala Leu Gln Arg Ala Ile Arg Leu Leu Asn Lys Tyr Val Ala His  
                       380                    385                   390  
 Ser Gly Ile Gly Asp Arg Ser Val Ser Leu Ile Val Phe Leu Thr  
                       395                    400                   405  
 Asp Gly Lys Pro Thr Val Gly Glu Thr His Thr Leu Lys Ile Leu  
                       410                    415                   420  
 Asn Asn Thr Arg Glu Ala Ala Arg Gly Gln Val Cys Ile Phe Thr  
                       425                    430                   435  
 Ile Gly Ile Gly Asn Asp Val Asp Phe Arg Leu Leu Glu Lys Leu  
                       440                    445                   450  
 Ser Leu Glu Asn Cys Gly Leu Thr Arg Arg Val His Glu Glu Glu

	455	460	465
Asp Ala Gly Ser Gln Leu Ile Gly Phe Tyr Asp Glu Ile Arg Thr			
470	475	480	
Pro Leu Leu Ser Asp Ile Arg Ile Asp Tyr Pro Pro Ser Ser Val			
485	490	495	
Val Gln Ala Thr Lys Thr Leu Phe Pro Asn Tyr Phe Asn Gly Ser			
500	505	510	
Glu Ile Ile Ile Ala Gly Lys Leu Val Asp Arg Lys Leu Asp His			
515	520	525	
Leu His Val Glu Val Thr Ala Ser Asn Ser Lys Lys Phe Ile Ile			
530	535	540	
Leu Lys Thr Asp Val Pro Val Arg Pro Gln Lys Ala Gly Lys Asp			
545	550	555	
Val Thr Gly Ser Pro Arg Pro Gly Gly Asp Gly Glu Gly Asp Thr			
560	565	570	
Asn His Ile Glu Arg Leu Trp Ser Tyr Leu Thr Thr Lys Glu Leu			
575	580	585	
Leu Ser Ser Trp Leu Gln Ser Asp Asp Glu Pro Glu Lys Glu Arg			
590	595	600	
Leu Arg Gln Arg Ala Gln Ala Leu Ala Val Ser Tyr Arg Phe Leu			
605	610	615	
Thr Pro Phe Thr Ser Met Lys Leu Arg Gly Pro Val Pro Arg Met			
620	625	630	
Asp Gly Leu Glu Glu Ala His Gly Met Ser Ala Ala Met Gly Pro			
635	640	645	
Glu Pro Val Val Gln Ser Val Arg Gly Ala Gly Thr Gln Pro Gly			
650	655	660	
Pro Leu Leu Lys Lys Pro Tyr Gln Pro Arg Ile Lys Ile Ser Lys			
665	670	675	
Thr Ser Val Asp Gly Asp Pro His Phe Val Val Asp Phe Pro Leu			
680	685	690	
Ser Arg Leu Thr Val Cys Phe Asn Ile Asp Gly Gln Pro Gly Asp			
695	700	705	
Ile Leu Arg Leu Val Ser Asp His Arg Asp Ser Gly Val Thr Val			
710	715	720	
Asn Gly Glu Leu Ile Gly Ala Pro Ala Pro Pro Asn Gly His Lys			
725	730	735	
Lys Gln Arg Thr Tyr Leu Arg Thr Ile Thr Ile Leu Ile Asn Lys			
740	745	750	
Pro Glu Arg Ser Tyr Leu Glu Ile Thr Pro Ser Arg Val Ile Leu			
755	760	765	
Asp Gly Gly Asp Arg Leu Val Leu Pro Cys Asn Gln Ser Val Val			
770	775	780	
Val Gly Ser Trp Gly Leu Glu Val Ser Val Ser Ala Asn Ala Asn			
785	790	795	
Val Thr Val Thr Ile Gln Gly Ser Ile Ala Phe Val Ile Leu Ile			
800	805	810	
His Leu Tyr Lys Lys Pro Ala Pro Phe Gln Arg His His Leu Gly			
815	820	825	
Phe Tyr Ile Ala Asn Ser Glu Gly Leu Ser Ser Asn Cys His Gly			
830	835	840	
Leu Leu Gly Gln Phe Leu Asn Gln Asp Ala Arg Leu Thr Glu Asp			
845	850	855	
Pro Ala Gly Pro Ser Gln Asn Leu Thr His Pro Leu Leu Leu Gln			
860	865	870	
Val Gly Glu Gly Pro Glu Ala Val Leu Thr Val Lys Gly His Gln			
875	880	885	
Val Pro Val Val Trp Lys Gln Arg Lys Ile Tyr Asn Gly Glu Glu			
890	895	900	
Gln Ile Asp Cys Trp Phe Ala Arg Asn Asn Ala Ala Lys Leu Ile			
905	910	915	

Asp	Gly	Glu	Tyr	Lys	Asp	Tyr	Leu	Ala	Ser	His	Pro	Phe	Asp	Thr
					920				925				930	
Gly	Met	Thr	Leu	Gly	Arg	Gly	Met	Ser	Arg	Glu	Leu			
					935				940					

## (2) INFORMATION FOR SEQ ID NO: 2:

## (i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 3636 base pairs
- (B) TYPE: nucleic acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

## (vii) IMMEDIATE SOURCE:

- (A) LIBRARY: UTRSNOT02
- (B) CLONE: 688183

## (xi) SEQUENCE DESCRIPTION: SEQ ID NO: 2 :

CCCTGAGAGC	GTCCCGCAGT	GGCTGGAGCC	CTGGGCGCTG	CAAACGTGTC	CCGCCGGGTC	60
CCCGAGCGTC	CCGCGCCCTC	GCCCCGCCAT	GCTCCTGCTG	CTGGGGCTGT	GCCTGGGCT	120
GTCCCTGTGT	GTGGGGTCGC	AGGAAGAGGC	GCAGAGCTGG	GGCCACTCTT	CGGAGCAGGA	180
TGGACTCAGG	GTCCCGAGGC	AAGTCAGACT	GTTGCAGAGG	CTGAAAACCA	AACCTTGAT	240
GACAGAATTG	TCAGTGAAGT	CTACCATCAT	TTCCCCTTAT	GCCTTCACTA	CGGTTTCCTG	300
CAGAATGCTG	AACAGAGCTT	CTGAAGACCA	GGACATTGAG	TTCCAGATGC	AGATTCCAGC	360
TGCAGCTTTC	ATCACCAACT	TCACTATGCT	TATTGGAGAC	AAGGTGTATC	AGGGCGAAAT	420
TACAGAGAGA	GAAAAGAAGA	GTGGTGATAG	GGTAAAAGAG	AAAAGGAATA	AAACCACAGA	480
AGAAAATGGA	GAGAAGGGGA	CTGAAATATT	CAGAGCTTCT	GCAGTGATTC	CCAGCAAGGA	540
CAAAGCCGCC	TTTTCCCTGA	GTTATGAGGA	GCTTCTGCAG	AGGCGCCTGG	GCAAGTACGA	600
GCACAGCATT	AGCGTGCAGC	CCCAGCAGCT	GTCCGGGAGG	CTGAGCGTGG	ACGTGAATAT	660
CCTGGAGAGC	CGGGGCATCG	CATCCCTGGA	GGTGCTGCCG	CTTCACAACA	GCAGGCAGAG	720
GGGCAGTGGG	CGCGGGGAAG	ATGATTCTGG	GCCTCCCCCA	TCTACTGTCA	TTAACCAAAA	780
TGAAACATTG	GCCAACATAA	TTTTTAAACC	TACTGTAGTA	CAACAAGCCA	GGATTGCCCA	840
GAATGGAATT	TTGGGAGACT	TTATCATTAG	ATATGACGTC	AATAGAGAAC	AGAGCATTGG	900
GGACATCCAG	GTTCTAAATG	GCTATTGTTG	GCACACTTT	GCTCCTAAAG	ACCTCCTCC	960
TTTACCCAAG	AATGTGGTAT	TCGTGCTTGA	CAGCAGTGCT	TCTATGGTGG	GAACCAAAC	1020
CCGGCAGACC	AAGGATGCC	TCTTCACAAT	TCTCCATGAC	CTCCGACCCC	AGGACCGTTT	1080
CAGTATCATT	GGATTTCCA	ACCGGATCAA	AGTATGGAAG	GACCACTTGA	TATCAGTCAC	1140
TCCAGACAGC	ATCAGGGATG	GGAAAGTGT	CATTCAACAT	ATGTCACCCA	CTGGAGGCAC	1200
AGACATCAAC	GGGGCCCTGC	AGAGGGCCAT	CAGGCTCCTC	AACAAGTACG	TGGCCCACAG	1260
TGGCATTGGA	GACCGGAGCG	TGTCCCTCAT	CGTCTTCCTG	ACGGATGGGA	AGCCCACGGT	1320
CGGGGAGACG	CACACCCTCA	AGATCCTCAA	CAACACCCGA	GAGGCCGCC	GAGGCCAAGT	1380
CTGCATCTTC	ACCATTGGCA	TCGGCAACGA	CGTGGACTTC	AGGCTGCTGG	AGAAACTGTC	1440
GCTGGAGAAC	TGTGGCTCA	CACGGCGCGT	GCACGAGGAG	GAGGACGCAG	GCTCGCAGCT	1500
CATCGGGTTC	TACGATGAAA	TCAGGACCCC	GCTCCTCTCT	GACATCCGCA	TCGATTATCC	1560
CCCCAGCTCA	GTGGTGCAGG	CCACCAAGAC	CCTGTTCCCC	AACTACTTCA	ACGGCTCGGA	1620
GATCATCATT	GCGGGGAAGC	TTGGTGGACAG	GAAGCTGGAT	CACCTGCACG	TGGAGGTAC	1680
CGCCAGCAAC	AGTAAGAAAT	TCATCATCCT	GAAGACAGAT	GTGCCTGTGC	GGCCTCAGAA	1740
GGCAGGGAAA	GATGTCACAG	GAAGCCCCAG	GCCTGGAGGC	GATGGAGAGG	GGGACACCAA	1800
CCACATCGAG	CGTCTCTGGA	GCTACCTCAC	CACAAAGGAG	CTGCTGAGCT	CCTGGCTGCA	1860
AAGTGACGAT	GAACCGGAGA	AGGAGCGGCT	GCAGCAGCGG	GCCCAGGCC	TGGCTGTGAG	1920
CTACCGCTTC	CTCACTCCCT	TCACCTCCAT	GAAGCTGAGG	GGGCCGGTCC	CACGCATGGA	1980
TGGCCTGGAG	GAGGCCACG	GCATGTCGGC	TGCCATGGGA	CCCGAACCGG	TGGTGCAGAG	2040
CGTGCAGGGA	GCTGGCACGC	AGCCAGGGCC	TTTGCTCAAG	AAGCCATACC	AGCCAAGAAT	2100
TAAAATCTCT	AAAACATCAG	TGGATGGTGA	TCCCCACTTT	GTTGTGGATT	TCCCCCTGAG	2160
CAGACTCACC	GTGTGCTCA	ACATTGATGG	GCAGCCCGGG	GACATCCTCA	GGCTGGTCTC	2220
TGATCACAGG	GACTCTGGTG	TCACAGTGAA	CGGAGAGTTA	ATTGGGGCAC	CCGCCCCCTCC	2280
AAATGGCCAC	AAGAACACAGC	GCACTTACTT	GCGCACTATC	ACCATCCTCA	TCAACAAGCC	2340

AGAGAGATCT TATCTCGAGA TCACACCGAG CAGAGTCATC TTGGATGGTG GGGACAGACT 2400  
 GGTGCTCCCC TGCAACCAGA GTGTGGTGGT GGGGAGCTGG GGGCTGGAGG TGTCCGTGTC 2460  
 TGCCAACGCC AATGTCACCG TCACCACATCCA GGGCTCCATA GCCTTTGTCA TCCTCATCCA 2520  
 CCTCTACAAA AAGCCGGCGC CCTTCCAGCG ACACCACCTG GGTTTCTACA TTGCCAACAG 2580  
 CGAGGGCCTT TCCAGCAACT GCCACGGACT GCTGGGTCAAG TTCCTGAATC AGGATGCCAG 2640  
 ACTCACAGAA GACCCTGCAG GGCCCAGCCA GAACCTCACT CACCCTCTGC TCCTTCAGGT 2700  
 GGGAGAGGGG CCTGAGGCCG TCCTAACAGT GAAAGGCCAC CAAGTCCCAG TGGTCTGGAA 2760  
 GCAAAGGAAG ATTTACAACG GGGAAAGAGCA GATAGACTGC TGGTTTGCCA GGAACAATGC 2820  
 CGCCAAACTG ATTGACGGGG AGTACAAGGA TTACCTGGCA TCCCATCCAT TTGACACAGG 2880  
 GATGACACTT GGCGGGGAA TGTCCAGGGA GCTCTGAAGC TGGCAGCCTT AAAGATGCAA 2940  
 GTGCATGAAG GACAGTGATG TGGGGAGGCC GTGGGGCAGC TCTTTTCATG GCTTGTACAC 3000  
 GCCTCAGCTC CTGGCAATTG GCTGGACTCC ATGACCCACC CCTGGTGCAG CATAGATCCG 3060  
 ACGTCTGTCT GGGCGAAGGG TAGGGGTGGG TAGGGGCCGG AAGCCTGAGT GCAAATGTCA 3120  
 TTTCCCTCTA CTGCCTCTTC CTGCCTCTCC CCACCCCTGCC CACATCCACA GAGGGGAGAG 3180  
 AAGGGTCATA GCTAAATGCA ACAAAAGTCTG TATCTTGTCC CAACCTGCTT TTCTGTTCTG 3240  
 TTAGCATATC ATAAAAGTAAG CCTTTCTGGT GAAGGAAGGT TGCTATGAAA CTTTTTTCT 3300  
 TGGTGGAAAT GCCCAAGTTT AGGCACTCTG CTTTTTGCC TACACTAATG CTTAGAAAGC 3360  
 TGTCTTTCA GTGGTGTTCG AGCCCCCAGA TGTGTGGCCA ACCTCTGCTG CAAAGGAATC 3420  
 TCTGCTGAG TCCAGGCCAC CAATCAGGCA AATAGCCCAT ACATTGATC GTTGTAAACC 3480  
 ATGAAGTCCTT TTCTTGCAAG ACGTTTTCT TCTGCTGTGG TATCTTGCCC TTAAAAAATTA 3540  
 GTTTTCATTA AAAAGAAATT TGATTGAAAA TTAAAAAAA ATAAAAAAA AAGAAAAAAA 3600  
 AAAAGAAAGA AAAAATAAAA AAAAAAAA AAAAAA 3636

## (2) INFORMATION FOR SEQ ID NO: 3:

## (i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 946 amino acids
- (B) TYPE: amino acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

## (vii) IMMEDIATE SOURCE:

- (A) LIBRARY: GENE BANK
- (B) CLONE: gi33985

## (xi) SEQUENCE DESCRIPTION: SEQ ID NO: 3 :

Met	Lys	Arg	Leu	Thr	Cys	Phe	Phe	Ile	Cys	Phe	Phe	Leu	Ser	Glu
														15
5														
Val	Ser	Gly	Phe	Glu	Ile	Pro	Ile	Asn	Gly	Leu	Ser	Glu	Phe	Val
														30
20														
Asp	Tyr	Glu	Asp	Leu	Val	Glu	Leu	Ala	Pro	Gly	Lys	Phe	Gln	Leu
														45
35														
Val	Ala	Glu	Asn	Arg	Arg	Tyr	Gln	Arg	Ser	Leu	Pro	Gly	Glu	Ser
														60
50														
Glu	Glu	Met	Met	Glu	Glu	Val	Asp	Gln	Val	Thr	Leu	Tyr	Ser	Tyr
														75
65														
Lys	Val	Gln	Ser	Thr	Ile	Thr	Ser	Arg	Met	Ala	Thr	Thr	Met	Ile
														90
80														
Gln	Ser	Lys	Val	Val	Asn	Asn	Ser	Pro	Gln	Pro	Gln	Asn	Val	Val
														105
95														
Phe	Asp	Val	Gln	Ile	Pro	Lys	Gly	Ala	Phe	Ile	Ser	Asn	Phe	Ser
														120
110														
Met	Thr	Val	Asp	Gly	Lys	Thr	Phe	Arg	Ser	Ser	Ile	Lys	Glu	Lys
														135
125														
Thr	Val	Gly	Arg	Ala	Leu	Tyr	Ala	Gln	Ala	Arg	Ala	Lys	Gly	Lys
														150
140														
Thr	Ala	Gly	Leu	Val	Arg	Ser	Ser	Ala	Leu	Asp	Met	Glu	Asn	Phe
														165
155														
Arg	Thr	Glu	Val	Asn	Val	Leu	Pro	Gly	Ala	Lys	Val	Gln	Phe	Glu

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 Z

	170		175		180									
Leu	His	Tyr	Gln	Glu	Val	Lys	Trp	Arg	Lys	Leu	Gly	Ser	Tyr	Glu
	185					190								195
His	Arg	Ile	Tyr	Leu	Gln	Pro	Gly	Arg	Leu	Ala	Lys	His	Leu	Glu
	200					205								210
Val	Asp	Val	Trp	Val	Ile	Glu	Pro	Gln	Gly	Leu	Arg	Phe	Leu	His
	215					220								225
Val	Pro	Asp	Thr	Phe	Glu	Gly	His	Phe	Asp	Gly	Val	Pro	Val	Ile
	230					235								240
Ser	Lys	Gly	Gln	Gln	Lys	Ala	His	Val	Ser	Phe	Lys	Pro	Thr	Val
	245					250								255
Ala	Gln	Gln	Arg	Ile	Cys	Pro	Ser	Cys	Arg	Glu	Thr	Ala	Val	Asp
	260					265								270
Gly	Glu	Leu	Val	Val	Leu	Tyr	Asp	Val	Lys	Arg	Glu	Glu	Lys	Ala
	275					280								285
Gly	Glu	Leu	Glu	Val	Phe	Asn	Gly	Tyr	Phe	Val	His	Phe	Phe	Ala
	290					295								300
Pro	Asp	Asn	Leu	Asp	Pro	Ile	Pro	Lys	Asn	Ile	Leu	Phe	Val	Ile
	305					310								315
Asp	Val	Ser	Gly	Ser	Met	Trp	Gly	Val	Lys	Met	Lys	Gln	Thr	Val
	320					325								330
Glu	Ala	Met	Lys	Thr	Ile	Leu	Asp	Asp	Leu	Arg	Ala	Glu	Asp	His
	335					340								345
Phe	Ser	Val	Ile	Asp	Phe	Asn	Gln	Asn	Ile	Arg	Thr	Trp	Arg	Asn
	350					355								360
Asp	Leu	Phe	Gln	Leu	Gln	Lys	His	Arg	Leu	Gln	Ile	Ala	Lys	Arg
	365					370								375
Tyr	Ile	Glu	Lys	Ile	Gln	Pro	Ser	Gly	Gly	Thr	Asn	Ile	Asn	Glu
	380					385								390
Ala	Leu	Leu	Arg	Ala	Ile	Phe	Ile	Leu	Asn	Glu	Ala	Asn	Asn	Leu
	395					400								405
Gly	Leu	Leu	Asp	Pro	Asn	Ser	Val	Ser	Leu	Ile	Ile	Leu	Val	Ser
	410					415								420
Asp	Gly	Asp	Pro	Thr	Val	Gly	Glu	Leu	Lys	Leu	Ser	Lys	Ile	Gln
	425					430								435
Lys	Asn	Val	Lys	Glu	Asn	Ile	Gln	Asp	Asn	Ile	Ser	Leu	Phe	Ser
	440					445								450
Leu	Gly	Met	Gly	Phe	Asp	Val	Asp	Tyr	Asp	Phe	Leu	Lys	Arg	Leu
	455					460								465
Ser	Asn	Glu	Asn	His	Gly	Ile	Ala	Gln	Arg	Ile	Tyr	Gly	Asn	Gln
	470					475								480
Asp	Thr	Ser	Ser	Gln	Leu	Lys	Lys	Phe	Tyr	Asn	Gln	Val	Ser	Thr
	485					490								495
Pro	Leu	Leu	Arg	Asn	Val	Gln	Phe	Asn	Tyr	Pro	His	Thr	Ser	Val
	500					505								510
Thr	Asp	Val	Thr	Gln	Asn	Asn	Phe	His	Asn	Tyr	Phe	Gly	Gly	Ser
	515					520								525
Glu	Ile	Val	Val	Ala	Gly	Lys	Phe	Asp	Pro	Ala	Lys	Leu	Asp	Gln
	530					535								540
Ile	Glu	Ser	Val	Ile	Thr	Ala	Thr	Ser	Ala	Asn	Thr	Gln	Leu	Val
	545					550								555
Leu	Glu	Thr	Leu	Ala	Gln	Met	Asp	Asp	Leu	Gln	Asp	Phe	Leu	Ser
	560					565								570
Lys	Asp	Lys	His	Ala	Asp	Pro	Asp	Phe	Thr	Arg	Lys	Leu	Trp	Ala
	575					580								585
Tyr	Leu	Thr	Ile	Asn	Gln	Leu	Leu	Ala	Glu	Arg	Ser	Leu	Ala	Pro
	590					595								600
Thr	Ala	Ala	Ala	Lys	Arg	Arg	Ile	Thr	Arg	Ser	Ile	Leu	Gln	Met
	605					610								615
Ser	Leu	Asp	His	His	Ile	Val	Thr	Pro	Leu	Thr	Ser	Leu	Val	Ile
	620					625								630

Glu Asn Glu Ala Gly Asp Glu Arg Met Leu Ala Asp Ala Pro Pro  
 635 640 645  
 Gln Asp Pro Ser Cys Cys Ser Gly Ala Leu Tyr Tyr Gly Ser Lys  
 650 655 660  
 Val Val Pro Asp Ser Thr Pro Ser Trp Ala Asn Pro Ser Pro Thr  
 665 670 675  
 Pro Val Ile Ser Met Leu Ala Gln Gly Ser Gln Val Leu Glu Ser  
 680 685 690  
 Thr Pro Pro Pro His Val Met Arg Val Glu Asn Asp Pro His Phe  
 695 700 705  
 Ile Ile Tyr Leu Pro Lys Ser Gln Lys Asn Ile Cys Phe Asn Ile  
 710 715 720  
 Asp Ser Glu Pro Gly Lys Ile Leu Asn Leu Val Ser Asp Pro Glu  
 725 730 735  
 Ser Gly Ile Val Val Asn Gly Gln Leu Val Gly Ala Lys Lys Pro  
 740 745 750  
 Asn Asn Gly Lys Leu Ser Thr Tyr Phe Gly Lys Leu Gly Phe Tyr  
 755 760 765  
 Phe Gln Ser Glu Asp Ile Lys Ile Glu Ile Ser Thr Glu Thr Ile  
 770 775 780  
 Thr Leu Ser His Gly Ser Ser Thr Phe Ser Leu Ser Trp Ser Asp  
 785 790 795  
 Thr Ala Gln Val Thr Asn Gln Arg Val Gln Ile Ser Val Lys Lys  
 800 805 810  
 Glu Lys Val Val Thr Ile Thr Leu Asp Lys Glu Met Ser Phe Ser  
 815 820 825  
 Val Leu Leu His Arg Val Trp Lys Lys His Pro Val Asn Val Asp  
 830 835 840  
 Phe Leu Gly Ile Tyr Ile Pro Pro Thr Asn Lys Phe Ser Pro Lys  
 845 850 855  
 Ala His Gly Leu Ile Gly Gln Phe Met Gln Glu Pro Lys Ile His  
 860 865 870  
 Ile Phe Asn Glu Arg Pro Gly Lys Asp Pro Glu Lys Pro Glu Ala  
 875 880 885  
 Ser Met Glu Val Lys Gly Gln Lys Leu Ile Ile Thr Arg Gly Leu  
 890 895 900  
 Gln Lys Asp Tyr Arg Thr Asp Leu Val Phe Gly Thr Asp Val Thr  
 905 910 915  
 Cys Trp Phe Val His Asn Ser Gly Lys Gly Phe Ile Asp Gly His  
 920 925 930  
 Tyr Lys Asp Tyr Phe Val Pro Gln Leu Tyr Ser Phe Leu Lys Arg  
 935 940 945  
 Pro

## (2) INFORMATION FOR SEQ ID NO: 4:

- (i) SEQUENCE CHARACTERISTICS:
  - (A) LENGTH: 911 amino acids
  - (B) TYPE: amino acid
  - (C) STRANDEDNESS: single
  - (D) TOPOLOGY: linear

- (vii) IMMEDIATE SOURCE:
  - (A) LIBRARY: GENEBAK
  - (B) CLONE: gi33989

- (xi) SEQUENCE DESCRIPTION: SEQ ID NO: 4 :

Met	Asp	Gly	Ala	Met	Gly	Pro	Arg	Gly	Leu	Leu	Leu	Cys	Met	Tyr
				5					10					15
Leu	Val	Ser	Leu	Leu	Ile	Leu	Gln	Ala	Met	Pro	Ala	Leu	Gly	Ser
				20					25					30
Ala	Thr	Gly	Arg	Ser	Lys	Ser	Ser	Glu	Lys	Arg	Gln	Ala	Val	Asp
				35					40					45
Thr	Ala	Val	Asp	Gly	Val	Phe	Ile	Arg	Ser	Leu	Lys	Val	Asn	Cys
				50					55					60
Lys	Val	Thr	Ser	Arg	Phe	Ala	His	Tyr	Val	Val	Thr	Ser	Gln	Val
				65					70					75
Val	Asn	Thr	Ala	Asn	Glu	Ala	Arg	Glu	Val	Ala	Phe	Asp	Leu	Glu
				80					85					90
Ile	Pro	Lys	Thr	Ala	Phe	Ile	Ser	Asp	Phe	Ala	Val	Thr	Ala	Asp
				95					100					105
Gly	Asn	Ala	Phe	Ile	Gly	Asp	Ile	Lys	Asp	Lys	Val	Thr	Ala	Trp
				110					115					120
Lys	Gln	Tyr	Arg	Lys	Ala	Ala	Ile	Ser	Gly	Glu	Asn	Ala	Gly	Leu
				125					130					135
Val	Arg	Ala	Ser	Gly	Arg	Thr	Met	Glu	Gln	Phe	Thr	Ile	His	Leu
				140					145					150
Thr	Val	Asn	Pro	Gln	Ser	Lys	Val	Thr	Phe	Gln	Leu	Thr	Tyr	Glu
				155					160					165
Glu	Val	Leu	Lys	Arg	Asn	His	Met	Gln	Tyr	Glu	Ile	Val	Ile	Lys
				170					175					180
Val	Lys	Pro	Lys	Gln	Leu	Val	His	His	Phe	Glu	Ile	Asp	Val	Asp
				185					190					195
Ile	Phe	Glu	Pro	Gln	Gly	Ile	Ser	Lys	Leu	Asp	Ala	Gln	Ala	Ser
				200					205					210
Phe	Leu	Pro	Lys	Glu	Leu	Ala	Ala	Gln	Thr	Ile	Lys	Lys	Ser	Phe
				215					220					225
Ser	Gly	Lys	Lys	Gly	His	Val	Leu	Phe	Arg	Pro	Thr	Val	Ser	Gln
				230					235					240
Gln	Gln	Ser	Cys	Pro	Thr	Cys	Ser	Thr	Ser	Leu	Leu	Asn	Gly	His
				245					250					255
Phe	Lys	Val	Thr	Tyr	Asp	Val	Thr	Arg	Asp	Glu	Ile	Cys	Asp	Leu
				260					265					270
Leu	Val	Ala	Asn	Asn	His	Phe	Ala	His	Phe	Phe	Ala	Pro	Gln	Asn
				275					280					285
Leu	Thr	Asn	Met	Asn	Lys	Asn	Val	Val	Phe	Val	Ile	Asp	Ile	Ser
				290					295					300
Gly	Ser	Met	Arg	Gly	Gln	Lys	Val	Lys	Gln	Thr	Lys	Glu	Ala	Leu
				305					310					315
Leu	Lys	Ile	Leu	Gly	Asp	Met	Gln	Pro	Gly	Asp	Tyr	Phe	Asp	Leu
				320					325					330
Val	Leu	Phe	Gly	Thr	Arg	Val	Gln	Ser	Trp	Lys	Gly	Ser	Leu	Val
				335					340					345
Gln	Ala	Ser	Glu	Ala	Asn	Leu	Gln	Ala	Ala	Gln	Asp	Phe	Val	Arg
				350					355					360
Gly	Phe	Ser	Leu	Asp	Glu	Ala	Thr	Asn	Leu	Asn	Gly	Gly	Leu	Leu
				365					370					375
Arg	Gly	Ile	Glu	Ile	Leu	Asn	Gln	Val	Gln	Glu	Ser	Leu	Pro	Glu
				380					385					390
Leu	Ser	Asn	His	Ala	Ser	Ile	Leu	Ile	Met	Leu	Thr	Asp	Gly	Asp
				395					400					405
Pro	Thr	Glu	Gly	Val	Thr	Asp	Arg	Ser	Gln	Ile	Leu	Lys	Asn	Val
				410					415					420
Arg	Asn	Ala	Ile	Arg	Gly	Arg	Phe	Pro	Leu	Tyr	Asn	Leu	Gly	Phe
				425					430					435
Gly	His	Asn	Val	Asp	Phe	Asn	Phe	Leu	Glu	Val	Met	Ser	Met	Glu
				440					445					450
Asn	Asn	Gly	Arg	Ala	Gln	Arg	Ile	Tyr	Glu	Asp	His	Asp	Ala	Thr

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	455		460		465									
Gln	Gln	Leu	Gln	Gly	Phe	Tyr	Ser	Gln	Val	Ala	Lys	Pro	Leu	Leu
					470				475					480
Val	Asp	Val	Asp	Leu	Gln	Tyr	Pro	Gln	Asp	Ala	Val	Leu	Ala	Leu
					485				490					495
Thr	Gln	Asn	His	His	Lys	Gln	Tyr	Tyr	Glu	Gly	Ser	Glu	Ile	Val
					500				505					510
Val	Ala	Gly	Arg	Ile	Ala	Asp	Asn	Lys	Gln	Ser	Ser	Phe	Lys	Ala
					515				520					525
Asp	Val	Gln	Ala	His	Gly	Glu	Gly	Gln	Glu	Phe	Ser	Ile	Thr	Cys
					530				535					540
Leu	Val	Asp	Glu	Glu	Glu	Met	Lys	Lys	Leu	Leu	Arg	Glu	Arg	Gly
					545				550					555
His	Met	Leu	Glu	Asn	His	Val	Glu	Arg	Leu	Trp	Ala	Tyr	Leu	Thr
					560				565					570
Ile	Gln	Glu	Leu	Leu	Ala	Lys	Arg	Met	Lys	Val	Asp	Arg	Glu	Val
					575				580					585
Arg	Ala	Asn	Leu	Ser	Ser	Gln	Ala	Leu	Arg	Met	Ser	Leu	Asp	Tyr
					590				595					600
Gly	Phe	Val	Thr	Pro	Leu	Thr	Ser	Met	Ser	Ile	Arg	Gly	Met	Ala
					605				610					615
Asp	Gln	Asp	Gly	Leu	Lys	Pro	Thr	Ile	Asp	Lys	Pro	Ser	Glu	Asp
					620				625					630
Ser	Pro	Pro	Leu	Glu	Met	Leu	Gly	Pro	Arg	Arg	Thr	Phe	Val	Leu
					635				640					645
Ser	Ala	Leu	Gln	Pro	Ser	Pro	Thr	His	Ser	Ser	Ser	Asn	Thr	Gln
					650				655					660
Arg	Leu	Pro	Asp	Arg	Val	Thr	Gly	Val	Asp	Thr	Asp	Pro	His	Phe
					665				670					675
Ile	Ile	His	Val	Pro	Gln	Lys	Glu	Asp	Thr	Leu	Cys	Phe	Asn	Ile
					680				685					690
Asn	Glu	Glu	Pro	Gly	Val	Ile	Leu	Ser	Leu	Val	Gln	Asp	Pro	Asn
					695				700					705
Thr	Gly	Phe	Ser	Val	Asn	Gly	Gln	Leu	Ile	Gly	Asn	Lys	Ala	Arg
					710				715					720
Ser	Pro	Gly	Gln	His	Asp	Gly	Thr	Tyr	Phe	Gly	Arg	Leu	Gly	Ile
					725				730					735
Ala	Asn	Pro	Ala	Thr	Asp	Phe	Gln	Leu	Glu	Val	Thr	Pro	Gln	Asn
					740				745					750
Ile	Thr	Leu	Asn	Pro	Gly	Phe	Gly	Gly	Pro	Val	Phe	Ser	Trp	Arg
					755				760					765
Asp	Gln	Ala	Val	Leu	Arg	Gln	Asp	Gly	Val	Val	Val	Thr	Ile	Asn
					770				775					780
Lys	Lys	Arg	Asn	Leu	Val	Val	Ser	Val	Asp	Asp	Gly	Gly	Thr	Phe
					785				790					795
Glu	Val	Val	Leu	His	Arg	Val	Trp	Lys	Gly	Ser	Ser	Val	His	Gln
					800				805					810
Asp	Phe	Leu	Gly	Phe	Tyr	Val	Leu	Asp	Ser	His	Arg	Met	Ser	Ala
					815				820					825
Arg	Thr	His	Gly	Leu	Leu	Gly	Gln	Phe	Phe	His	Pro	Ile	Gly	Phe
					830				835					840
Glu	Val	Ser	Asp	Ile	His	Pro	Gly	Ser	Asp	Pro	Thr	Lys	Pro	Asp
					845				850					855
Ala	Thr	Met	Val	Val	Arg	Asn	Arg	Arg	Leu	Thr	Val	Thr	Arg	Gly
					860				865					870
Leu	Gln	Lys	Asp	Tyr	Ser	Lys	Asp	Pro	Trp	His	Gly	Ala	Glu	Val
					875				880					885
Ser	Cys	Trp	Phe	Ile	His	Asn	Asn	Gly	Ala	Gly	Leu	Ile	Asp	Gly
					890				895					900
Ala	Tyr	Thr	Asp	Tyr	Ile	Val	Pro	Asp	Ile	Phe				
					905				910					

(2) INFORMATION FOR SEQ ID NO: 5:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 885 amino acids
- (B) TYPE: amino acid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(vi) IMMEDIATE SOURCE:

(A) LIBRARY: GENE BANK  
(B) CLONE: gi288563

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 5 :

Met	Val	Ala	Leu	Ser	His	Leu	Gly	Ser	Ala	Leu	Gln	Leu	Gly	Ser
					5					10				15
Leu	Cys	Phe	Pro	Arg	Ser	Pro	Phe	Arg	Leu	Leu	Gly	Lys	Arg	Ser
					20					25				30
Leu	Pro	Glu	Gly	Val	Ala	Asn	Gly	Ile	Glu	Val	Tyr	Ser	Thr	Lys
					35					40				45
Ile	Asn	Ser	Lys	Val	Thr	Ser	Arg	Phe	Ala	His	Asn	Val	Val	Thr
					50					55				60
Met	Arg	Ala	Val	Asn	Arg	Ala	Asp	Thr	Ala	Lys	Glu	Val	Ser	Phe
					65					70				75
Asp	Val	Glu	Leu	Pro	Lys	Thr	Ala	Phe	Ile	Thr	Asn	Phe	Thr	Leu
					80					85				90
Thr	Ile	Asp	Gly	Val	Thr	Tyr	Pro	Gly	Asn	Val	Lys	Glu	Lys	Glu
					95					100				105
Val	Ala	Lys	Lys	Gln	Tyr	Glu	Lys	Ala	Val	Ser	Gln	Gly	Lys	Thr
					110					115				120
Ala	Gly	Leu	Val	Lys	Ala	Ser	Gly	Arg	Lys	Leu	Glu	Lys	Phe	Thr
					125					130				135
Val	Ser	Val	Asn	Val	Ala	Ala	Gly	Ser	Lys	Val	Thr	Phe	Glu	Leu
					140					145				150
Thr	Tyr	Glu	Glu	Leu	Leu	Lys	Arg	His	Lys	Gly	Lys	Tyr	Glu	Met
					155					160				165
Tyr	Leu	Lys	Val	Gln	Pro	Lys	Gln	Leu	Val	Lys	His	Phe	Glu	Ile
					170					175				180
Glu	Val	Asp	Ile	Phe	Glu	Pro	Gln	Gly	Ile	Ser	Met	Leu	Asp	Ala
					185					190				195
Glu	Ala	Ser	Phe	Ile	Thr	Asn	Asp	Leu	Leu	Gly	Ser	Ala	Leu	Thr
					200					205				210
Lys	Ser	Phe	Ser	Gly	Lys	Lys	Gly	His	Val	Ser	Phe	Lys	Pro	Ser
					215					220				225
Leu	Asp	Gln	Gln	Arg	Ser	Cys	Pro	Thr	Cys	Thr	Asp	Ser	Leu	Leu
					230					235				240
Asn	Gly	Asp	Phe	Thr	Ile	Thr	Tyr	Asp	Val	Asn	Arg	Glu	Ser	Pro
					245					250				255
Gly	Asn	Val	Gln	Ile	Val	Asn	Gly	Tyr	Phe	Val	His	Phe	Phe	Ala
					260					265				270
Pro	Gln	Gly	Leu	Pro	Val	Val	Pro	Lys	Asn	Val	Ala	Phe	Val	Ile
					275					280				285
Asp	Ile	Ser	Gly	Ser	Met	Ala	Gly	Arg	Lys	Leu	Glu	Gln	Thr	Lys
					290					295				300
Glu	Ala	Leu	Leu	Arg	Ile	Leu	Glu	Asp	Met	Lys	Glu	Glu	Asp	Tyr
					305					310				315
Leu	Asn	Phe	Ile	Leu	Phe	Ser	Gly	Asp	Val	Ser	Thr	Trp	Lys	Glu
					320					325				330

His	Leu	Val	Gln	Ala	Thr	Pro	Glu	Asn	Leu	Gln	Glu	Ala	Arg	Thr
				335					340					345
Phe	Val	Lys	Ser	Met	Glu	Asp	Lys	Gly	Met	Thr	Asn	Ile	Asn	Asp
				350					355					360
Gly	Leu	Leu	Arg	Gly	Ile	Ser	Met	Leu	Asn	Lys	Ala	Arg	Glu	Glu
				365					370					375
His	Arg	Ile	Pro	Glu	Arg	Ser	Thr	Ser	Ile	Val	Ile	Met	Leu	Thr
				380					385					390
Asp	Gly	Asp	Ala	Asn	Val	Gly	Glu	Ser	Arg	Pro	Glu	Lys	Ile	Gln
				395					400					405
Glu	Asn	Val	Arg	Asn	Ala	Ile	Gly	Gly	Lys	Phe	Pro	Leu	Tyr	Asn
				410					415					420
Leu	Gly	Phe	Gly	Asn	Asn	Leu	Asn	Tyr	Asn	Phe	Leu	Glu	Asn	Met
				425					430					435
Ala	Leu	Glu	Asn	His	Gly	Phe	Ala	Arg	Arg	Ile	Tyr	Glu	Asp	Ser
				440					445					450
Asp	Ala	Asp	Leu	Gln	Leu	Gln	Gly	Phe	Tyr	Glu	Glu	Val	Ala	Asn
				455					460					465
Pro	Leu	Leu	Thr	Gly	Val	Glu	Met	Glu	Tyr	Pro	Glu	Asn	Ala	Ile
				470					475					480
Leu	Asp	Leu	Thr	Gln	Asn	Thr	Tyr	Gln	His	Phe	Tyr	Asp	Gly	Ser
				485					490					495
Glu	Ile	Val	Val	Ala	Gly	Arg	Leu	Val	Asp	Glu	Asp	Met	Asn	Ser
				500					505					510
Phe	Lys	Ala	Asp	Val	Lys	Gly	His	Gly	Ala	Thr	Asn	Asp	Leu	Thr
				515					520					525
Phe	Thr	Glu	Glu	Val	Asp	Met	Lys	Glu	Met	Glu	Lys	Ala	Leu	Gln
				530					535					540
Glu	Arg	Asp	Tyr	Ile	Phe	Gly	Asn	Tyr	Ile	Glu	Arg	Leu	Trp	Ala
				545					550					555
Tyr	Leu	Thr	Ile	Glu	Gln	Leu	Leu	Glu	Lys	Arg	Lys	Asn	Ala	His
				560					565					570
Gly	Glu	Glu	Lys	Glu	Asn	Leu	Thr	Ala	Arg	Ala	Leu	Asp	Leu	Ser
				575					580					585
Leu	Lys	Tyr	His	Phe	Val	Thr	Pro	Leu	Thr	Ser	Met	Val	Val	Thr
				590					595					600
Lys	Pro	Glu	Asp	Asn	Glu	Asp	Glu	Arg	Ala	Ile	Ala	Asp	Lys	Pro
				605					610					615
Gly	Glu	Asp	Ala	Glu	Ala	Thr	Pro	Val	Ser	Pro	Ala	Met	Ser	Tyr
				620					625					630
Leu	Thr	Ser	Tyr	Gln	Pro	Pro	Gln	Asn	Pro	Tyr	Tyr	Tyr	Val	Asp
				635					640					645
Gly	Asp	Pro	His	Phe	Ile	Ile	Gln	Ile	Pro	Glu	Lys	Asp	Asp	Ala
				650					655					660
Leu	Cys	Phe	Asn	Ile	Asp	Glu	Ala	Pro	Gly	Thr	Val	Leu	Arg	Leu
				665					670					675
Ile	Gln	Asp	Ala	Val	Thr	Gly	Leu	Thr	Val	Asn	Gly	Gln	Ile	Thr
				680					685					690
Gly	Asp	Lys	Arg	Gly	Ser	Pro	Asp	Ser	Lys	Thr	Arg	Lys	Thr	Tyr
				695					700					705
Phe	Gly	Lys	Leu	Gly	Ile	Arg	Asn	Ala	Gln	Met	Asp	Phe	Gln	Val
				710					715					720
Glu	Val	Thr	Thr	Glu	Lys	Ile	Thr	Cys	Gly	Thr	Gly	Arg	Ala	Ser
				725					730					735
Thr	Phe	Ser	Trp	Leu	Asp	Thr	Val	Thr	Val	Thr	Gln	Asp	Gly	Leu
				740					745					750
Ser	Met	Met	Ile	Asn	Arg	Lys	Asn	Met	Val	Val	Ser	Phe	Gly	Asp
				755					760					765
Gly	Val	Thr	Phe	Val	Val	Val	Leu	His	Gln	Val	Trp	Lys	Lys	His
				770					775					780
Pro	Val	His	Arg	Asp	Phe	Leu	Gly	Phe	Tyr	Val	Val	Asp	Ser	His

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785		790		795
Arg Met Ser Ala Gln Thr His Gly Leu	Leu	Gly Gln Phe Phe Gln		
800		805		810
Pro Phe Asp Phe Lys Val Ser Asp Ile Arg Pro Gly Ser Asp Pro				
815		820		825
Thr Lys Pro Asp Ala Thr Leu Val Val Lys Asn His Gln Leu Ile				
830		835		840
Val Thr Arg Gly Ser Gln Lys Asp Tyr Arg Lys Asp Ala Ser Ile				
845		850		855
Gly Thr Lys Val Val Cys Trp Phe Val His Asn Asn Gly Glu Gly				
860		865		870
Leu Ile Asp Gly Val His Thr Asp Tyr Ile Val Pro Asn Leu Phe				
875		880		885

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